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AN ABSTRACT OF THE SCIENTIFIC EXHIBIT

URINE AND THE GROUND SUBSTANCE

By Joseph Seifter, M.D.; A. H. Butt, M.D.; and E. A. Hauser, Ph.D.

Shown at the Annual Convention of the American Medical Association
New York City, June 1 to 5, 1953

Properties common to both urine and the ground substance of tissues were the subject of this exhibit. These common properties provide the basis for an understanding of a possible mechanism of action for hyaluronidase in the management of renal lithiasis.*

What Is Ground Substance?

"The ground substance is the mucoid cement material present between the cells and fibrils of the mesenchyme . . . The ground substance is practically everywhere in the body in the same way as mortar is present between the bricks of a house. The ground substance is both a barrier protecting cells from dangerous outside agencies and a conductor making available to cells the metabolites indispensable for cell life and removing waste products."

Significance of the Resemblance of Some of the Properties of Urine to Those of Ground Substance

"The mucopolysaccharide hyaluronic acid is a characteristic component of ground substance and is largely responsible for [its] state of viscosity . . . As a result of our studies on ground substance, we have come to look upon ground substance, not as an inert barrier, but as a dynamic material which participates in the spreading and transport of materials between cells. This spreading and transport activity is always enhanced by hyaluronidase and almost always inhibited or delayed by stress, ACTH and cortisone.

"Hyaluronic acid seems to play the same role in urine as it does in intracellular spaces in making possible the transport of metabolites and inorganic ions for elimination. Without the 'protective colloid' and dispersing action of hyaluronic acid, precipitation and crystallization occur.

"The most critical role played by hyaluronic acid is probably in the tubules where a tremendous concentration of urine is occurring and where precipitation in the absence of 'protective colloid' should occur in accordance with von Weimarn's law.

"The subcutaneous administration of an adequate dose of hyaluronidase results in an increase of 'protective colloids' in the urine. This is associated with an increase in total reducing substances and in glucuronic acid . . ."

"On the other hand, stress or the administration of cortisone or ACTH results in crystalluria that is indicative of suppression of 'protective colloid' action . . ."

Summary of Results Following Administration of Hyaluronidase for Renal Lithiasis

Group	Stone Formation Controlled	Stone Formation Slowed	Stones Smaller and Less Dense	Stone Formation Not Controlled
A—15 Cases Primary† Stones	9(60.00%)	3(20.00%)	1(6.67%)	2(13.33%)
B—21 Cases Secondary Stones	7(33.33%)	7(33.33%)	1(4.77%)	6(28.57%)

*The only hyaluronidase used in the study reported in this exhibit was the Wyeth brand, WYDASE®.

†No associated infection or mechanical obstruction. All other stones are considered secondary.

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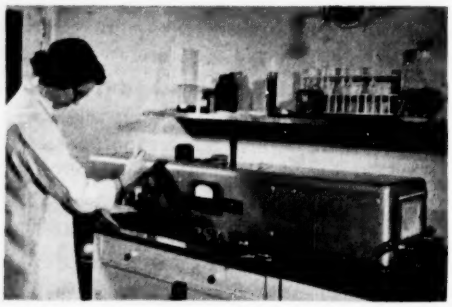

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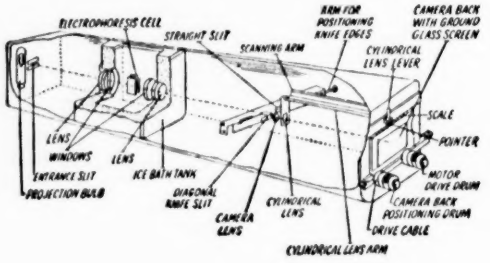

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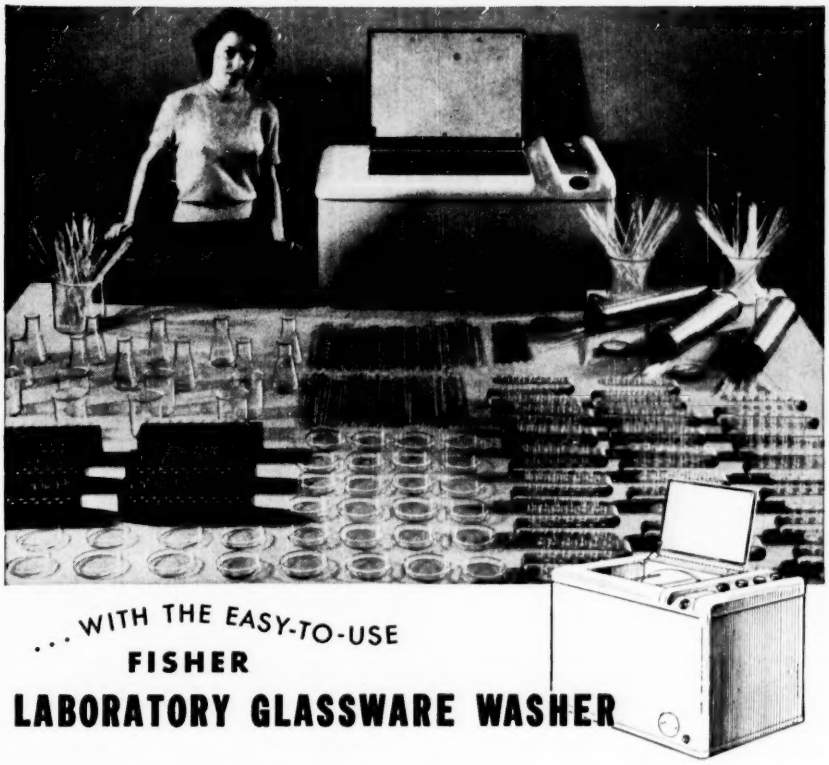

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ANTIBODY RESPONSE TO NON-STREPTOCOCCAL ANTIGENS AS RELATED TO RHEUMATIC FEVER SUSCEPTIBILITY[1]

By JOSEPH M. MILLER,[2] SIDNEY KIBRICK,[3] AND BENEDICT F. MASSELL

(From the House of the Good Samaritan and the Research Division of Infectious Diseases, Children's Medical Center, Boston, Mass.)

(Submitted for publication March 2, 1953; accepted April 7, 1953)

It is now well established that practically all initial attacks and subsequent recurrences of rheumatic fever are precipitated by antecedent Group A hemolytic streptococcal infection. Evidence to support this concept has been based on the close epidemiologic and bacteriologic association of acute rheumatic fever and streptococcal infection (1–9) as well as on the striking similarity of the antibody responses in both of these conditions (10). Despite this dependence of the rheumatic state upon prior hemolytic streptococcal infection, only a small portion of those exposed to this group of organisms actually develop rheumatic fever. Such disparity in the disease incidence indicates the importance of the host factor and suggests that susceptibility variables are important determinants of pathogenesis of rheumatic fever.

The purpose of this report is to explore the possibility that there exists in subjects with rheumatic fever a peculiarity of immune response which predisposes to the rheumatic state. Others have shown that hemolytic streptococcal respiratory infection complicated by rheumatic fever is associated with higher mean antistreptococcal antibody titers than is the uncomplicated illness (11, 12), and the conclusion from such data has been that an immunologic hyperreactivity in such susceptible individuals may be fundamental for the development of rheumatic fever. Our studies have aimed to determine whether such supposed immunologic hyperreactivity of rheumatic subjects is confined to the streptococcus alone or whether one may also detect a more general augmentation of antibody response to non-streptococcal antigens as well. Preliminary results of such studies have been presented elsewhere (13). The present report is an extension of these earlier data.

MATERIALS AND METHOD

Influenza immunization: In preliminary experiments, influenza virus vaccine (Lederle) was used as a non-streptococcal test antigen. Nine rheumatic fever patients hospitalized at the House of the Good Samaritan were selected for immunization, and eighteen children hospitalized for miscellaneous orthopedic conditions at the Children's Medical Center served as controls. All of the rheumatic fever subjects were in the convalescent stage of the disease and all had either an organic heart murmur or definite chorea. The rheumatic and control subjects were of comparable ages. Each child was given a single intradermal injection of 0.1 cc. of a 1:2.5 dilution of influenza virus vaccine containing influenza A PR8 and influenza B Lee strains. Blood specimens were obtained on all subjects immediately prior to the immunizing injection and at periodic intervals thereafter. The antibody response to these antigens was determined by both the hemagglutinin-inhibition and the complement-fixation techniques. Maximum antibody levels, reached within four weeks after immunization, provided the basis for comparison of the response in the two groups tested. Antihemagglutinins were determined by the pattern method (14) using chicken erythrocytes, and complement fixing antibodies were determined by the method of Enders and Levens (15). The antigen suspensions for use in these tests were prepared in the allantoic sacs of ten day old chick embryos from PR8 and Lee B inocula supplied by Dr. J. F. Enders. After two days at 35°C. the viruses were harvested and stored in the frozen state until needed.

Typhoid immunization: In a second series of experiments, fourteen children with rheumatic heart disease, hospitalized at the House of the Good Samaritan, were given a single 0.1 cc. intradermal dose of monovalent typhoid vaccine containing 100 million heat-killed organisms. As controls, ten children of comparable age with Legg-Perthes disease, hospitalized at the New England Peabody Home for Crippled Children, were similarly immunized. These children showed no evidence of rheumatic fever either by history or by physical examination. Other than mild local discomfort at the site of injection, there were no temperature elevations, no elevations in sedimentation rate, nor other untoward reactions in any of the subjects. In addition to a control blood sample

[1] Supported by a grant from the Helen Hay Whitney Foundation.

[2] Research Fellow, New England Chapter, Arthritis and Rheumatism Foundation, 1950–51.

[3] Post-doctorate Fellow, United States Public Health Service, 1950.

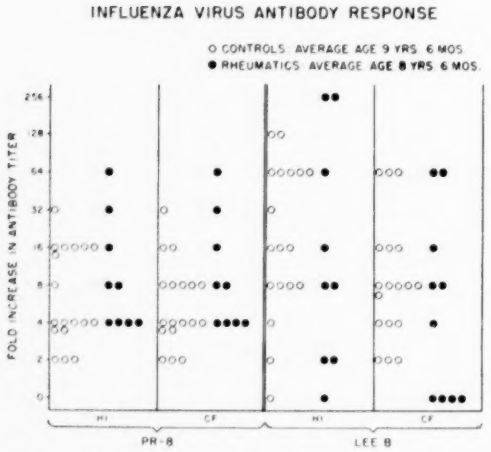

FIG. 1. COMPARISON OF PEAK RESPONSE TO INFLUENZA IMMUNIZATION USING HEMAGGLUTINATION INHIBITION (HI) AND COMPLEMENT FIXATION (CF) ANTIBODY MEASUREMENT

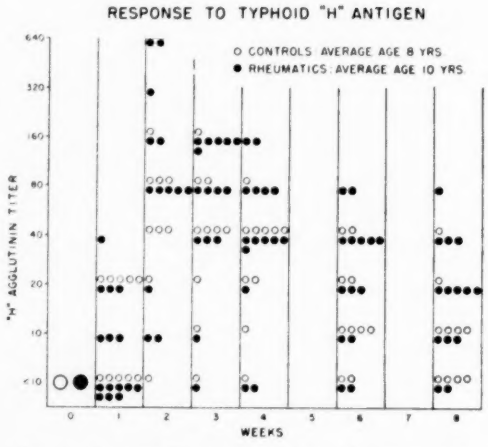

FIG. 2. SCATTER DIAGRAM OF ANTI-H AGGLUTININ TITERS FOLLOWING TYPHOID IMMUNIZATION

taken on each subject prior to the immunizing injection, serial blood samples were drawn at weekly intervals over the next four weeks and at bi-weekly intervals for four additional weeks. Agglutinin titers were measured against both the H and O antigens. Antigen material used in the tests was prepared from strains of typhoid organisms obtained from the Department of Bacteriology of the Harvard Medical School. A standard serial dilution technique was used for antibody assay (16).

RESULTS

Influenza immunization: Since many of the subjects in both the rheumatic fever and control groups showed moderately high antibody levels prior to immunization, the antibody response attained after immunization has been plotted in terms of the fold increase produced by immunization (Figure 1). The scatter diagram in Figure 1 indicates that there was no significant difference in grouping of the antibody responses achieved by rheumatic and control subjects. Both groups of subjects showed a wide variation of response to influenza virus vaccine as reflected by the antibodies measured. Titers varied from no change following immunization to instances of a 256-fold increase.

A presentation of antibody data in terms of fold increase omits consideration of the effect of the pre-immunization titer on the final level attained. In general, the higher the initial titer, the less the fold increase following immunization. In Table I, therefore, there are presented composite pre- and post-immunization data from the same patients in terms of geometric mean antibody levels along with the titer fold increases as determined from these data. This table indicates that, despite the low mean pre-immunization titers of the rheumatic subjects, their antibody rise was, in general, less than that of the controls.

Typhoid immunization: Results of individual antibody levels following typhoid immunization have been plotted in the scatter diagrams of Figures 2 and 3. In the case of the anti-H agglutinin,

TABLE I

Geometric mean antibody titers against influenza PR8 and Lee B

		Anti-hemagglutinins against PR8			C. F. antibodies against PR8			Anti-hemagglutinins against Lee B			C. F. antibodies against Lee B		
	No. of cases	Before	After	Fold rise	Before	After	Fold rise	Before	After	Fold rise	Before	After	Fold rise
Rheumatics	9	17.3	138	8.0	7.4	32.0	4.3	7.4	74.7	10.1	3.4	20.2	5.9
Controls	18	35.9	237	6.6	9.3	52.8	5.7	8.3	155.2	18.7	3.7	33.3	9.0

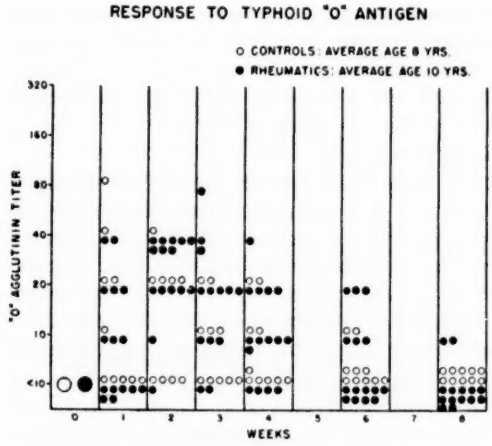

FIG. 3. SCATTER DIAGRAM OF ANTI-O AGGLUTININ TITERS FOLLOWING TYPHOID IMMUNIZATION

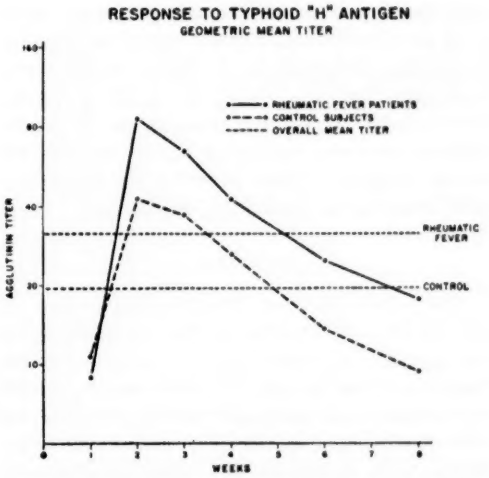

FIG. 4. GEOMETRIC MEAN TITERS AT WEEKLY INTERVALS AND OVERALL MEAN TITER OF H-AGGLUTININS FOLLOWING TYPHOID IMMUNIZATION

peak levels of response were reached after two weeks in both the rheumatic subjects and in the controls. As in the influenza studies, there is much overlapping in Figure 2 between the two groups with no clear cut difference in antibody response shown by the rheumatic fever patients. Similarly with the anti-O agglutinin (Figure 3) there was extensive overlapping of individual subjects of both groups. While the general height of antibody level attained for the O-agglutinin was moderately lower than the H-agglutinin for both groups, no distinct difference between the groups was discernible from the scatter diagram representation of the data.

The individual results were then tabulated by geometric means for each week after immunization, and these means are indicated in Figures 4 and 5. In Figure 4, the H-agglutinin level of the rheumatic group of subjects over the course of eight weeks now appears higher than that of the

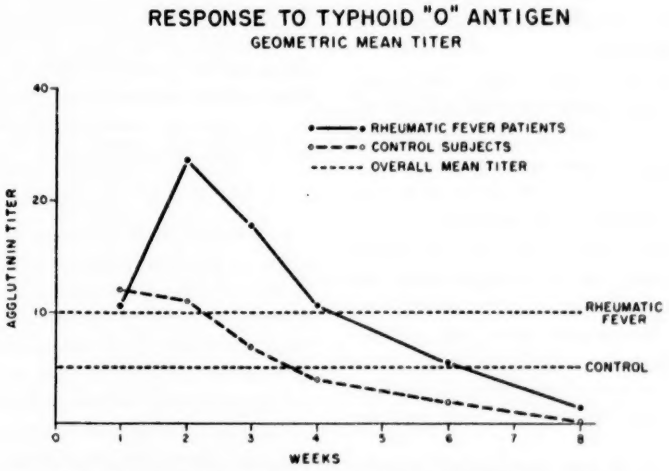

FIG. 5. GEOMETRIC MEAN TITERS AT WEEKLY INTERVALS AND OVERALL MEAN TITER OF O-AGGLUTININS FOLLOWING TYPHOID IMMUNIZATION

control subjects. Likewise, the geometric mean of all the observations without regard for time interval after immunization is higher for the rheumatic fever patients. In Figure 5, the geometric means of the O-agglutinin titers for the rheumatic subjects were also somewhat higher than for the control subjects. These overall mean differences were found to be statistically significant.[4]

DISCUSSION

The results of these immunization studies do not confirm the hypothesis that rheumatic fever subjects show a general hyperreactivity of immune response, at least as judged by their response to the non-streptococcal antigenic stimuli of influenza virus and typhoid vaccine. In the instance of influenza virus immunization, initially elevated antibody levels prior to immunization may have obscured true differences of response from a uniform baseline, and for this reason typhoid immunization was considered to be a more valid method of studying these young subjects in the unsensitized state. None of the particular children immunized gave a history of previous typhoid infection or typhoid immunization, and baseline agglutinin levels confirmed the absence of typhoid antibodies. Although statistically significant, the observed difference between the mean titers is rather small and clinical conclusions of general hyperreactivity of rheumatic subjects appear hazardous. Also, the relatively small number of cases studied may hold for these particular individuals and not for rheumatic subjects in general.

A recent report by Creger, Choy, and Rantz (17) in which similar immunization studies, using mismatched blood, resulted in more striking increases of antibody response by rheumatic subjects warrants comment. Of the three rheumatic subjects demonstrating hyperreactivity of response to heterologous blood cells, the one subject showing the greatest rise in isoagglutinins had received a previous injection of mismatched blood and hence the response may have been, in part at least, the manifestation of a "booster phenomenon." Further, the use of mismatched blood in persons who already demonstrate isoagglutinins to heterologous blood substances prior to challenge suggests that the criterion of an unsensitized state does not truly exist. Thus, even the first dose of group B cells given to a group A individual represents a "booster challenge" to the production of isoagglutinins.

On the other hand, the use of such antigenic boosters in previously sensitized persons may be the more appropriate means of detecting immunologic hyperreactions especially as related to hypersensitivity theories of rheumatic fever pathogenesis. If, as many have postulated, rheumatic fever develops in susceptible individuals only after repeated exposure to the hemolytic streptococcus, the experimental results of Creger, Choy, and Rantz are corroborating evidence. The work of Murphy and Swift (18) in animals is also in accord with such reasoning. It could follow that reimmunization of our subjects with repeated doses of typhoid vaccine might then result in more striking differences of antibody response and that such booster stimuli by the same antigen might ultimately demonstrate a more significant augmentation of immune response in rheumatic fever subjects. In the absence of such data and on the basis of our results with influenza and typhoid vaccines, however, it seems unwarranted to attribute any striking hyperreactivity of response to these antigens in the rheumatic subjects tested.

Such discrepancies of antibody response in somewhat comparable studies emphasize the lack of a uniform pattern of host hyperreactivity in rheumatic fever. Increased numbers of observations using varied measurements of immunologic indices, will be necessary before it is possible to define the role of immune responsiveness in rheumatic fever susceptibility.

SUMMARY

1. Twenty-three children in the convalescent stage of rheumatic fever were immunized with either polyvalent influenza virus vaccine or with monovalent typhoid vaccine. Twenty-eight nonrheumatic children of comparable age were similarly immunized and served as controls.

2. Results of influenza immunization, using complement fixing and hemagglutination inhibiting antibodies as indices of response, showed a wide variation in titer with no essential difference between the two groups.

[4] Statistical analyses of these experiments were performed by Dr. William E. Reynolds, Assistant Professor of Preventive Medicine, Harvard Medical School.

3. Whereas the geometric mean antibody to typhoid vaccine was slightly higher in rheumatic fever patients, there was extensive overlapping and scattering of individual responses in both groups over the eight week period of study.

4. On the basis of these observations using both a viral and bacterial antigen, it seems unwarranted to attribute to these rheumatic individuals any general hyperreactivity of immune response as an explanation for the development of the rheumatic state.

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THE EFFECTS OF CARBON DIOXIDE INHALATION UPON THE CEREBRAL BLOOD FLOW AND CEREBRAL OXYGEN CONSUMPTION IN VASCULAR DISEASE [1]

By PAUL NOVACK,[2] HENRY A. SHENKIN, LEONARD BORTIN, BERNARD GOLUBOFF, AND ALVIN M. SOFFE WITH THE TECHNICAL ASSISTANCE OF MRS. PETER BATSON AND MISS DORIS GOLDEN

(From the Departments of Neurosurgery, Research, and Medicine of the Albert Einstein Medical Center, Southern Division, Phila., Penna.)

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Alterations of the cerebral circulation were for a long time presumed to reflect principally changes in the systemic circulation. Recently, a high degree of control of the cerebral blood flow has been demonstrated to be the result of intrinsic mechanisms. An important means of such intrinsic control results from the effects of changes in arterial partial pressure of carbon dioxide upon the cerebral vessels. An increased arterial partial pressure of carbon dioxide, such as is produced by the inhalation of 5 per cent carbon dioxide, results in a marked and selective cerebral vasodilatation in normal young individuals with a consequent increase in the cerebral blood flow (1). It appeared that the effect of the inhalation of carbon dioxide might provide a convenient tool for the study of the degree of vascular tone in cerebrovascular disease states. The present investigation was initiated with the purpose of using this method to gain insight into the relative contributions of vascular spasm and arteriosclerosis towards the production of an increased cerebrovascular resistance.

METHODS

The cerebral blood flow (CBF) was measured by the nitrous oxide technique (2). All patients were in the postabsorptive state and the recumbent position. Following the completion of a control flow, an interval of 15 minutes was allowed to elapse. The patient was then primed for five minutes with a gas mixture of the following composition: 5 per cent CO_2, 21 per cent O_2, 74 per cent N_2. The mask was then rapidly switched to allow the patient to inhale a similar gas mixture, containing 15 per cent N_2O and 59 per cent N_2 in addition to 5 per cent CO_2 and 21 per cent O_2. In this manner the experimental flow was determined over a ten minute period. The jugular venous pressure (JVP) was measured with a spinal fluid manometer using the level at which the vessel was entered as the "O" reference point. In the supine position, this is approximately at the level of the right atrium. The mean arterial blood pressure (MABP) was measured in a femoral artery using a damped mercury manometer. The oxygen and carbon dioxide contents of the blood samples were determined by the method of van Slyke and Neill (3). Blood pH was determined anaerobically at room temperature with a glass electrode and corrected to body temperature using the formula of Rosenthal (4). The pCO_2 was determined from standard nomograms (3).

MATERIAL

The subjects of this study have been divided into four groups according to criteria previously described (5): 1) Young normotensive individuals without evidence of arteriosclerosis with a mean age of 30; 2) elderly, arteriosclerotic but normotensive individuals with a mean age of 64; 3) hypertensive individuals without evidence of arteriosclerosis with a mean age of 51; and, 4) hypertensive and arteriosclerotic individuals whose mean age was 55. Six of the latter group had histories of cerebrovascular accidents or mental deterioration. The presence of arteriosclerosis was diagnosed on the basis of historical or electrocardiographic evidence of coronary artery disease, vascular calcifications on X-ray, peripheral vascular ischemia, retinal changes, or a previous cerebrovascular accident. The control data for these and other groups have been presented and discussed in detail in another communication (5). The present report is concerned only with the responses to CO_2 inhalation.

RESULTS

Table IA presents the cerebral circulatory data for Group 1. A 40 per cent increase in the CBF from the control value of 53 cc. per 100 gm. per min. to 74 cc. per 100 gm. per min. is noted. This increased blood flow is in part attributable to the 4 per cent increase in MABP but is mainly a reflection of the 33 per cent drop in CVR. The increase in JVP noted is a reflection of the increased

[1] Supported by a grant from the National Institutes of Health.

[2] National Heart Institute Postdoctorate Research Fellow, 1952–1953.

TABLE IA

*Effects of 5 per cent CO_2 inhalation on cerebral circulatory functions of young, normotensive, non-arteriosclerotic individuals**

Subject	CBF *cc./100 gm./min.*		CVR *mm. Hg/cc./ 100 gm./min.*		$CMRO_2$ *cc./100 gm./min.*		MABP *mm. Hg*		JVP *mm. H_2O*		R. Q.	
	I	II	I	II	I	II	I	II	I	II	I	II
G. C.	47	54	1.8	1.7	2.8	2.3	99	105	186	195	1.00	0.92
S. W.	77	89	1.0	0.9	4.2	2.3	84	90	90	88	0.93	0.78
F. B.	47	64	1.5	1.2	3.4	2.9	79	85	101	100	0.88	1.09
S. G.	35	48	2.1	1.6	2.5	2.5	81	85	98	126	0.97	0.96
M. H.	22	81	3.9	1.0	1.6	3.5	91	92	84	166	0.99	0.94
D. M.	54	78	1.3	0.9	2.9	3.6	79	82	98	130	1.29	1.16
S. R.	38	80	2.5	1.3	2.0	2.7	100	105	73	85	0.78	1.10
A. H.	52	64	2.0	1.7	4.8	3.8	111	111	61	67	1.06	0.95
S. K.	74	77	1.1	1.0	4.9	4.4	95	97	152	220	1.01	0.93
E. G.	54	61	1.7	1.4	2.7	2.1	100	94	108	112	0.92	1.04
S. D.	78	102	1.1	0.9	5.1	3.3	91	104	101	128	0.92	0.92
D. C.	57	92	1.3	0.9	3.8	3.8	83	90	80	100	0.98	1.00
Mean	53	74†	1.8	1.2†	3.4	3.4	91	95†	103	126†	0.98	0.98

* CBF: Cerebral blood flow.

CVR: Cerebrovascular resistance $= \frac{MABP - JVP}{CBF}$.

$CMRO_2$: Cerebral oxygen consumption.
MABP: Mean arterial blood pressure.
JVP: Jugular venous pressure.
R.Q.: Cerebral respiratory quotient.
I: Control value.
II: Value after inhalation of 5% CO_2.

† Significant change, $p < .05$.

CBF. The other cerebral circulatory functions are not significantly changed.

Table IB presents in detail the effects of the inhalation of 5 per cent CO_2 upon the various blood constituents.

Table IIA presents the data concerned with the effects of 5 per cent CO_2 inhalation upon the cerebral circulatory functions of the aged, arteriosclerotic, but normotensive group. The increase in CBF from 47 cc. per 100 gm. per min. to 55 cc.

TABLE IB

*Effects of inhalation of 5 per cent CO_2 on blood constituents of young, normotensive, non-arteriosclerotic individuals**

Subject	AO_2 *Vol. %*		ACO_2 *Vol. %*		ApH		$ApCO_2$ *mm. Hg*		VO_2 *Vol. %*		VCO_2 *Vol. %*		VpH		$VpCO_2$ *mm. Hg*		$(A - V)O_2$ *Vol. %*	
	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II
G. C.	16.8	18.4	47.3	48.9	7.33	7.25	46	56	10.8	12.2	53.3	54.5	7.30	7.23	51	60	6.0	6.1
S. W.	14.9	15.3	54.4	55.3	7.33	7.29	51	56	9.4	9.4	9.4	59.9	7.30	7.28	55	60	5.4	5.9
F. B.	14.6	14.8	40.1	44.7	7.31	7.25	39	49	7.3	4.5	46.6	49.6	7.28	7.22	47	58	7.3	4.5
S. G.	19.4	19.9	40.8	43.6	7.36	7.33	38	43	12.4	14.7	47.7	48.6	7.33	7.30	40	47	7.1	5.3
M. H.	17.9	17.9	39.6	46.8	7.42	7.33	32	45	10.6	13.5	46.9	50.9	7.39	7.29	36	51	7.3	4.3
D. M.	18.8	19.3	46.8	49.4	7.29	7.29	47	52	12.3	14.7	55.3	54.7	7.28	7.28	57	59	6.6	4.6
S. R.	16.5	17.6	51.4	52.5	7.34	7.31	48	53	11.3	14.2	55.4	56.3	7.29	7.27	57	62	5.2	3.4
A. H.	15.4	15.1	46.6	51.0	7.39	7.34	40	48	6.3	9.2	56.3	56.6	7.39	7.33	48	53	9.1	6.0
S. K.	20.2	20.2	50.9	51.8	7.39	7.33	45	51	13.5	14.4	57.6	57.1	7.33	7.29	51	57	6.6	5.7
E. G.	15.0	14.9	50.9	52.2	7.33	7.30	48	52	10.0	11.3	55.5	55.8	7.29	7.27	54	60	5.0	3.5
S. P.	11.9	11.0	48.5	51.6	7.38	7.28	42	53	5.3	8.8	54.5	54.6	7.32	7.26	49	56	6.6	3.2
D. O.	12.1	12.1	48.2	51.0	7.41	7.35	37	45	5.4	8.0	54.7	55.1	7.35	7.32	46	50	6.7	4.1
Mean	16.1	16.4	47.1	49.9†	7.36	7.30†	43	50†	9.6	11.2†	53.6	54.5†	7.32	7.28†	49	56†	6.6	4.7†

* A: Arterial.
V: Venous.
O_2: Oxygen content.
CO_2: Carbon dioxide content.
pCO_2: Partial pressure of carbon dioxide.

† Significant change, $p < .05$.

TABLE IIA

*Effects of inhalation of 5 per cent CO_2 on cerebral circulatory functions of aged, normotensive, arteriosclerotic individuals**

Subject	CBF *cc./100 gm./min.*		CVR *mm. Hg/cc./ 100 gm./min.*		$CMRO_2$ *cc./100 gm./min.*		MABP *mm. Hg*		JVP *mm. H_2O*		R. Q.	
	I	II	I	II	I	II	I	II	I	II	I	II
M. D.	48	46	2.0	2.3	3.3	3.6	100	110	65	70	0.89	0.71
S. A.	29	39	3.2	2.4	1.5	1.7	99	100	75	64	1.12	0.78
H. S.	38	39	2.6	2.8	3.0	1.9	100	113	38	67	1.01	0.88
J. P.	98	99	0.8	0.8	6.4	3.9	77	79	59	67	0.51	0.81
E. G.	54	63	1.7	1.6	3.9	3.9	98	111	98	105	0.92	1.16
B. D.	45	50	1.8	1.6	2.8	2.5	95	92	156	180	0.81	0.96
E. G.	33	42	2.4	1.8	1.0	1.8	86	79	77	82	0.83	1.09
M. S.	36	33	2.6	3.0	2.2	1.5	100	108	108	115	0.97	0.84
S. K.	51	68	1.3	1.1	3.5	3.2	72	76	54	58	0.96	0.81
J. L.	42	66	2.7	1.7	3.3	3.6	115	115	60	60	0.99	0.98
Mean	47	55†	2.1	1.9	3.1	2.7	94	98	79	87	0.90	0.90

* See Tables IA and IB for definitions of symbols.
† Significant change, $p < .05$.

TABLE IIB

*Effects of inhalation of 5 per cent CO_2 on blood constituents of aged, normotensive, arteriosclerotic individuals**

Subject	AO_2 *Vol. %*		ACO_2 *Vol. %*		ApH		$ApCO_2$ *mm. Hg*		VO_2 *Vol. %*		VCO_2 *Vol. %*		VpH		$VpCO_2$ *mm. Hg*		$(A - V)O_2$ *Vol. %*	
	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II
M. D.	18.5	18.8	48.1	51.4	7.31	7.26	47	54	11.6	13.2	54.3	55.4	7.27	7.24	55	65	6.9	5.6
S. A.	18.4	18.8	44.8	47.5	7.34	7.30	43	50	13.4	14.3	50.4	50.9	7.30	7.28	53	56	5.0	4.5
H. S.	17.0	15.9	60.7	64.6	7.35	7.30	56	66	9.1	11.1	68.6	68.8	7.30	7.26	70	75	7.8	4.8
J. P.	15.1	15.1	50.0	51.8	—	—	—	—	8.6	11.2	55.3	55.0	—	—	—	—	6.5	3.9
E. G.	19.8	20.6	46.3	47.0	7.36	7.34	43	47	12.6	14.4	53.0	54.3	7.30	7.28	47	59	7.2	6.2
B. D.	18.1	19.5	43.6	46.1	—	—	—	—	12.0	14.5	48.5	50.8	—	—	—	—	6.1	5.0
E. Gi.	19.5	20.5	42.5	41.8	—	—	—	—	16.6	16.2	44.8	46.4	—	—	—	—	2.9	4.3
M. S.	19.2	20.0	48.6	52.0	7.31	7.29	49	57	13.5	15.4	54.5	55.9	7.29	7.25	57	65	5.7	4.6
S. K.	16.7	16.8	47.4	49.8	7.33	7.31	45	49	9.9	12.1	53.9	55.5	7.31	7.27	53	59	6.8	4.7
J. L.	16.5	16.8	44.5	47.5	7.39	7.32	38	47	8.8	11.3	52.1	52.9	7.33	7.29	46	52	7.7	5.5
Mean	17.9	18.3	47.7	50.0†	7.34	7.30†	46	53†	11.6	13.4†	54.3	55.4†	7.30	7.27†	54	62†	6.3	4.9†

* See Tables IA and IB for definitions of symbols.
† Significant change, $p < .05$.

TABLE IIIA

*Effects of inhalation of 5 per cent CO_2 on cerebral circulatory functions of individuals with essential hypertension uncomplicated by arteriosclerosis**

Subject	CBF *cc./100 gm./min.*		CVR *mm. Hg/cc./ 100 gm./min.*		$CMRO_2$ *cc./100 gm./min.*		MABP *mm. Hg*		JVP *mm. H_2O*		R. Q.	
	I	II	I	II	I	II	I	II	I	II	I	II
B. B.	50	72	2.5	1.9	3.1	1.2	133	151	120	145	0.98	0.95
G. B.	60	76	2.4	2.0	3.7	3.5	154	160	137	160	1.09	0.92
C. H.	47	82	3.2	2.3	3.3	4.9	160	200	160	200	0.77	0.96
S. P.	55	103	1.9	1.0	2.8	4.5	115	110	120	125	0.79	0.83
D. B.	67	78	1.9	1.7	3.7	3.4	132	140	107	107	0.81	0.86
R. S.	31	102	3.5	1.2	1.9	2.0	121	137	165	260	1.15	2.06
Mean	52	86†	2.6	1.7†	3.1	3.6	136	150†	135	166†	0.93	1.10

* See Tables IA and IB for definitions of symbols.
† Significant change, $p < .05$.

TABLE IIIB

*Effects of inhalation of 5 per cent CO_2 on blood constituents of individuals with essential hypertension uncomplicated by arteriosclerosis**

Subject	AO_2 Vol. %		ACO_2 Vol. %		ApH		$ApCO_2$ mm. Hg		VO_2 Vol. %		VCO_2 Vol. %		VpH		$VpCO_2$ mm. Hg		$(A-V)O_2$ Vol. %	
	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II
B. B.	15.2	15.5	48.6	50.6	7.44	7.43	37	40	9.1	11.1	54.6	54.9	7.35	7.34	47	59	6.1	4.5
G. B.	16.6	17.6	45.7	48.8	7.41	7.33	37	47	10.4	13.2	58.4	53.0	7.33	7.29	49	52	6.2	4.4
C. H.	16.5	17.0	54.9	56.4	7.38	7.33	47	54	9.5	11.0	60.3	62.1	7.35	7.32	52	62	7.1	6.0
S. P.	15.7	14.1	48.5	51.6	7.39	7.35	41	46	9.6	9.8	52.6	55.2	7.31	7.30	51	54	5.1	4.3
D. B.	16.2	16.4	52.2	53.1	7.41	7.34	42	49	10.7	12.0	56.6	56.9	7.33	7.32	51	52	5.5	4.4
R. S.	16.0	15.0	44.8	53.6	7.40	7.32	37	52	10.0	16.9	51.7	49.7	7.36	7.30	43	53	6.0	1.9
Mean	15.9	15.9	49.1	52.4†	7.41	7.35†	40	48†	9.9	12.3†	54.7	55.3†	7.34	7.31†	49	54	6.0	4.3

* See Tables IA and IB for definitions of symbols.
† Significant change, $p < .05$.

TABLE IVA

*Effects of inhalation of 5 per cent CO_2 on cerebral circulatory functions of hypertensive arteriosclerotic individuals**

Subject	CBF cc./100 gm./min.		CVR mm. Hg/cc./100 gm./min.		$CMRO_2$ cc./100 gm./min.		MABP mm. Hg		JVP mm. H_2O		R. Q.	
	I	II	I	II	I	II	I	II	I	II	I	II
M. S.	33	39	3.5	3.1	2.4	2.6	120	125	60	70	0.98	0.95
S. F.	33	46	3.9	2.6	2.6	1.6	132	130	54	130	1.05	0.80
E. S.	51	32	2.5	4.1	2.6	1.9	141	145	210	198	1.27	0.72
S. L.	41	94	3.0	1.4	2.8	5.2	123	133	59	71	0.88	1.02
S. S.	23	24	6.3	6.0	2.5	—	140	150	65	80	0.86	—
C. K.	29	48	4.9	3.6	2.6	2.6	143	172	35	49	0.99	0.90
S. G.	34	60	3.3	2.1	2.1	2.1	117	135	55	110	1.11	1.11
D. K.	33	46	3.4	2.0	2.6	2.2	120	100	100	114	0.88	0.93
J. L.	41	50	3.7	3.2	2.7	2.6	156	169	78	90	0.77	1.30
M. W.	44	54	2.6	2.1	3.7	3.1	125	128	145	170	0.93	0.80
Mean	36	49†	3.7	3.0†	2.6	2.7	132	139†	86	108†	0.97	0.90

* See Tables IA and IB for definitions of symbols.
† Significant change, $p < .05$.

TABLE IVB

*Effects of inhalation of 5 per cent CO_2 on blood constituents of hypertensive arteriosclerotic individuals**

Subject	AO_2 Vol. %		ACO_2 Vol. %		ApH		$ApCO_2$ mm. Hg		VO_2 Vol. %		VCO_2 Vol. %		VpH		$VpCO_2$ mm. Hg		$(A-V)O_2$ Vol. %	
	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II
M. S.	21.2	21.2	40.5	43.4	7.37	7.30	38	47	13.9	14.7	47.6	49.6	7.32	7.27	48	56	7.3	6.5
S. F.	16.8	14.8	45.6	53.8	—	—	—	—	8.8	11.4	53.8	56.5	—	—	—	—	8.0	3.4
E. S.	19.2	21.6	44.9	48.1	7.30	7.25	47	57	14.1	15.6	51.3	52.5	7.27	7.24	55	63	5.0	6.0
S. L.	19.0	19.1	48.1	49.1	7.38	7.36	42	47	12.3	13.5	54.1	54.8	7.33	7.33	52	53	6.8	5.6
S. S.	22.5	—	54.1	—	7.39	—	47	—	10.1	—	64.7	—	7.37	—	59	—	12.3	—
C. K.	19.1	18.4	37.8	47.8	7.51	7.33	22	46	10.2	13.5	46.6	52.3	7.41	7.33	38	54	8.9	5.0
S. G.	10.8	10.9	39.9	46.3	—	—	—	—	4.7	7.3	46.6	50.3	—	—	—	—	6.0	3.6
D. K.	17.4	17.3	43.8	49.7	7.50	7.36	27	45	9.6	12.4	50.7	54.2	7.44	7.32	39	52	7.8	4.8
J. L.	19.6	19.6	50.0	48.9	7.43	7.37	40	45	13.0	14.4	54.9	55.8	7.39	7.32	45	54	6.7	5.2
M. W.	19.2	18.9	49.1	51.5	7.41	7.37	41	46	10.9	13.2	56.9	56.1	7.37	7.32	47	54	8.4	5.8
Mean	18.5	18.0	45.4	48.7†	7.41	7.33†	38	48†	10.8	12.9†	52.7	53.6†	7.36	7.30†	48	55†	7.7	5.1†

* See Tables IA and IB for definitions of symbols.
† Significant change, $p < .05$.

per 100 gm. per min. is significant ($p < .05$) but is less than for Group 1 (17 per cent vs. 40 per cent). The increased CBF apparently results from the combination of an increase in the MABP from 94 mm. Hg to 98 mm. Hg and a slight drop in the CVR, from 2.1 mm. Hg per cc. per 100 gm. per min. to 1.9 mm. Hg per cc. per 100 gm. per min., although neither of these changes is statistically significant in itself. Table IIB summarizes the data for the effects of 5 per cent CO_2 inhalation upon the blood constituents of the individuals in this group. The changes noted with respect to blood CO_2 contents, pH, and pCO_2 are of the same magnitude as in Group 1.

Table IIIA presents the data illustrating the effects of 5 per cent CO_2 inhalation upon the cerebral circulation of hypertensive, non-arteriosclerotic individuals. The 65 per cent increase in CBF from 52 cc. per 100 gm. per min. to 86 cc. per 100 gm. per min. is greater, but not significantly so, than that noted for Group 1. This increased CBF is attributable to the combination of a 34 per cent drop in the CVR and a 10 per cent increase in the MABP. Table IIIB summarizes the blood constituent data for the group.

Table IVA presents cerebral circulatory data for the hypertensive, arteriosclerotic patients indicating the effects of 5 per cent CO_2 inhalation. On a percentage basis, the 36 per cent increase in the CBF from the greatly reduced control value is comparable to that noted for Group 1 (40 per cent) and somewhat less than that noted for Group 3 (65 per cent). However, the final level obtained is far below that noted in the two aforementioned groups. The 0.7 mm. Hg per cc. per 100 gm. per min. drop in CVR noted for this group is, in terms of absolute units, similar to the decreases in CVR described for Groups 1 and 3. This is, however, only a 19 per cent decrease from the control CVR, as compared to the 33 per cent and 34 per cent decreases in CVR for Groups 1 and 3 respectively. Table IVB summarizes the blood constituent data for Group 4. The increases in pCO_2 obtained with 5 per cent CO_2 inhalation are of the same order as noted for the previous groups.

DISCUSSION

Vascular resistance is principally a function of blood viscosity and the diameters of the blood channels, particularly the arteriolar tree. There is no reason to believe there were differences in blood viscosity among the various groups. Changes in vascular resistance caused by an increased arterial pCO_2 largely reflect variations in the diameters of the vessels. A diminished arteriolar diameter, with its consequent increased vascular resistance, may in turn be the result of either organic occlusive changes or functional vasoconstriction.

In considering the arteriosclerotic but normotensive group of elderly individuals, the control values for the various cerebral circulatory functions were found to be within normal limits. It was only when these patients were subjected to an increased arterial pCO_2 that significant abnormalities of their cerebral vasculatures were unmasked. Unlike the normal response, little decrease in CVR occurred with the elevation of the arterial pCO_2. The failure to obtain cerebrovascular relaxation in the presence of arteriosclerosis may be explained by several possibilities: 1) The vessels may be so rigid that they can neither dilate nor constrict to any stimulus; 2) the vessels in the individuals of this group may be already maximally dilated as a compensatory response to the development of arteriosclerosis; 3) arteriosclerotic vessels may no longer respond to the specific dilating stimulus of an increased arterial pCO_2. The finding that the CVR of patients having combined hypertension and arteriosclerosis (Group 4) may be lowered by CO_2 inhalations demonstrates that the cerebral vessels of arteriosclerotic individuals are sensitive to an increased arterial pCO_2 and that such vessels still have a residual elasticity. This favors the second of the above possibilities as an explanation for the failure of CO_2 inhalations to lower the CVR in the normotensive, arteriosclerotic group.

The possibility that CO_2 inhalation increases extracerebral contamination of internal jugular venous blood specimens has been considered as a factor which might influence the results. Study of the curves in the various groups failed to reveal evidence of such an occurrence in a significant degree. The ten minute equilibration period for nitrous oxide was found adequate in all groups both before and after CO_2 inhalation.

Various measures, including the inhalation of carbon dioxide, have been recommended to stimulate the cerebral blood flow in patients who have suffered a cerebral thrombosis. When the throm-

bosis is the result of arteriosclerotic narrowing in the absence of hypertension, it would seem from the results obtained in this study that such efforts would be doomed to failure. Furthermore, if such patients are in incipient or actual cardiac decompensation, inhalation of 5 per cent CO_2 has been found to embarrass further the general circulation and in many instances to decrease the cerebral blood flow (6). However, no final conclusion can be reached relative to the efficacy of such measures without a direct investigation of the cerebral circulation during the acute phase of a cerebral thrombosis. At such times cerebral vasoconstriction mediated by the autonomic system could presumably occur even in the absence of hypertension.

In uncomplicated hypertension, the increased cerebrovascular resistance is generally considered to be primarily the result of increased vascular tone rather than of arteriosclerotic narrowing of the vessels. Various measures which reduce the increased cerebrovascular resistance have been studied. Sympathectomy has been found to result in an 18 per cent decrease (7), high spinal anesthesia, in a 16 per cent decrease (8), and dihydroergocornine, in a 32 per cent decrease (9) in the cerebrovascular resistance in essential hypertension. In the present study, the inhalation of 5 per cent CO_2 produced a mean decrease in the cerebrovascular resistance of 35 per cent, revealing CO_2 to be as powerful a cerebral vasodilator in essential hypertension as any of the previously studied methods. In addition, it is to be noted that this reduction in CVR resulting from an increased arterial pCO_2 is not associated with a fall in the head of pressure as are the other methods. Even after the reduction in cerebrovascular resistance produced by the inhalation of 5 per cent CO_2, the cerebrovascular resistance of patients with essential hypertension was 1.7 mm. Hg per cc. per 100 gm. per min. as compared to the value of 1.2 mm. Hg per cc. per 100 gm. per min. for the "normal" group after CO_2 inhalation. This residual increased vascular resistance in the hypertensive group may be attributed to either a failure of CO_2 to produce maximal vasodilatation, or to the existence of clinically inapparent arteriosclerosis in this group, or, to a combination of the two. The failure of such measures as sympathectomy, high spinal anesthesia, or dihydroergocornine to produce a greater cerebrovascular relaxation than is obtained with CO_2 suggests that near maximal vasodilatation is obtained with CO_2 inhalation, and that the residual increased cerebrovascular resistance may be due to organic occlusive disease. The possibility of directly measuring the vascular resistance of an organ and roughly dissociating its components attributable to functional spasm and organic occlusion may offer a test for predicting the maximal efficacy of therapeutic measures designed to relieve increased vascular resistance.

The above concept may be applied to the interpretation of the data for the group of cases possessing hypertension and clinically apparent arteriosclerosis. Increase of the arterial pCO_2 in this group resulted in the lowering of the cerebrovascular resistance from 3.7 mm. Hg per cc. per 100 gm. per min. to 3.0 mm. Hg per cc. per 100 gm. per min. The high residual cerebrovascular resistance suggests that the arteriosclerosis present in this group is considerably greater than in any of the previously discussed groups. This is the same conclusion previously derived (5).

From these data there seems to emerge some clarification of the interrelated effects of hypertension and arteriosclerosis upon the cerebral hemodynamics. In hypertension uncomplicated by clinically evident arteriosclerosis, the cerebrovascular resistance is increased, mostly due to increased arteriolar tone, but in part probably due to arteriosclerosis. This increase in cerebrovascular resistance parallels the increase in arterial pressure and there is no reduction in the cerebral blood flow. In arteriosclerosis without hypertension, a normal cerebral blood flow and cerebrovascular resistance tend to be maintained, possibly by some relaxation of the normal vascular tone. Finally, where hypertension and arteriosclerosis coexist, the arteriosclerosis seems to be of a much greater severity and diffuseness than that occurring in the absence of hypertension, and an increased vascular tone persists. The net effect is a great increase in the cerebrovascular resistance and a marked reduction in the cerebral blood flow and oxygen consumption.

CONCLUSIONS

1. The effects of inhalation of 5 per cent CO_2 upon the cerebral circulations of individuals with varying degrees of vascular disease have been studied.

2. The normal response to an elevation of arterial pCO_2 is a slight increase in the mean ar-

terial blood pressure and a drop in cerebrovascular resistance with a consequent increase in cerebral blood flow.

3. A group composed of normotensive arteriosclerotic individuals failed to show a significant decrease of the cerebrovascular resistance, although the cerebral blood flow increased slightly.

4. A small group of individuals with uncomplicated essential hypertension were found to respond normally to CO_2 inhalation. The decrease in cerebrovascular resistance noted in this group was as great as had previously been produced with other methods.

5. A group of hypertensive arteriosclerotic patients also responded normally to CO_2 inhalation. The residual cerebrovascular resistance after CO_2 vasodilatation was still markedly elevated above normal.

6. It is suggested that the response to CO_2 inhalation provides a convenient means for dissociating and estimating the components of an increased cerebrovascular resistance attributable to functional vasoconstriction and arteriosclerotic narrowing to the lumens of the vessels.

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ESTIMATION OF THE RATE OF RELEASE OF ADRENAL 17-HYDROXYCORTICOSTEROIDS IN THE HUMAN BEING BY THE VENOUS CATHETER TECHNIQUE WITH A METHOD FOR DETERMINING PLASMA 17-HYDROXYCORTICOSTEROIDS [1]

By PHILIP K. BONDY [2] AND JOAN R. ALTROCK

(From the Departments of Medicine, Emory U. School of Medicine and Grady Memorial Hospital, and the Department of Internal Medicine, Yale U. School of Medicine, New Haven, Conn.)

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The importance of the role of the adrenal cortex in protein and carbohydrate metabolism and in adjustments to injury has been well established in experimental animals. Studies of the activity of the adrenal cortex in similar situations in human beings have been handicapped by lack of a direct objective method for estimating the rate of secretion of adrenal cortical hormones in the intact man. Since it seemed possible that the intravenous catheter technique might be applicable to this problem, attempts were made to estimate the rate of secretion of the adrenal hormones directly by obtaining blood from the left renal vein, proximal to the entrance of the adrenal vein, and multiplying the arterio-venous difference in concentration of cortical hormones by the renal plasma flow. The method has serious limitations, and, as will be discussed below, is probably not sufficiently accurate to be useful as an experimental clinical tool.

ANALYTICAL METHODS

At the time that this project was initiated, no specific method was available for the estimation of adrenal cortical steroids in plasma. Attempts were therefore made to use bioassay methods on whole plasma, since it was hoped that this would afford a high degree of specificity, without any danger of loss of hormone during the extractions and manipulations incidental to the use of a chemical assay. The concentration of liver-glycogen-depositing material in human arterial plasma proved to be too small to be detected. For the purposes of the assay, 70 mg. of glucose were dissolved in 1.26 ml. of plasma + 0.14 ml. of ethyl alcohol. This solution was injected subcutaneously in seven 0.2 ml. portions into each adrenalectomized mouse according to the schedule of Venning, Kasmin, and Bell (1). Eight to 10 mice were used for assaying each preparation. In all of the assays, the activity of the plasma was less than that of 0.1 ml. of Upjohn aqueous adrenal cortical extract [3] (equivalent to 20 μgm. of hydrocortisone). No significant difference could be found between the activity of plasma and saline. As a result of our unsuccessful attempts to apply this type of bioassay to the estimation of the plasma corticosteroid concentration, we have concluded that the quantity of these substances present in plasma is too small to be detected by the Venning technique. This finding is not in agreement with the observation of Paschkis, Cantarow, Walkling, and Boyle who were able to use this method for the measurement of adrenal corticoids in dog plasma (2).

Since bioassay was unsatisfactory, a method was devised for the purification of plasma extracts so that the phenylhydrazine colorimetric reaction of Porter and Silber could be applied (3). The method finally developed is shown in the flow sheet (Table I) and is described in detail below:

Plasma proteins are precipitated, and the lipids simultaneously extracted by adding 15 to 20 ml. of plasma to 20 times its volume of a 3:1 mixture of methanol and ether. The plasma is dropped slowly from a pipette into 200 ml. centrifuge bottles containing the methanol-ether, agitated by a magnetic stirrer. The stirrer is allowed to run two or three minutes after the precipitation is complete to insure complete extraction. The sample is then centrifuged for 15 minutes at moderate speed; the supernatant, which contains the plasma lipids as well as most of the water-soluble components, is transferred to a 2000 ml. Erlenmeyer flask, where it is evaporated to dryness under a stream of air at not more than 55° C. Evaporation to dryness must be thorough but not unduly prolonged. Hydrocortisone is not destroyed by exposure to large volumes of warm air provided that the pH is approximately neutral.

Several spatulas of granular tin metal are placed in the two-liter flask, in a separatory funnel, and in each of two heavy duty 40-ml. centrifuge tubes. The residue of the plasma extract is dissolved in two successive 10 ml. portions of 10 per cent isopropyl alcohol at pH 1 (H_2SO_4) which are pooled in the separatory funnel, where they are extracted with two 10 ml. portions of

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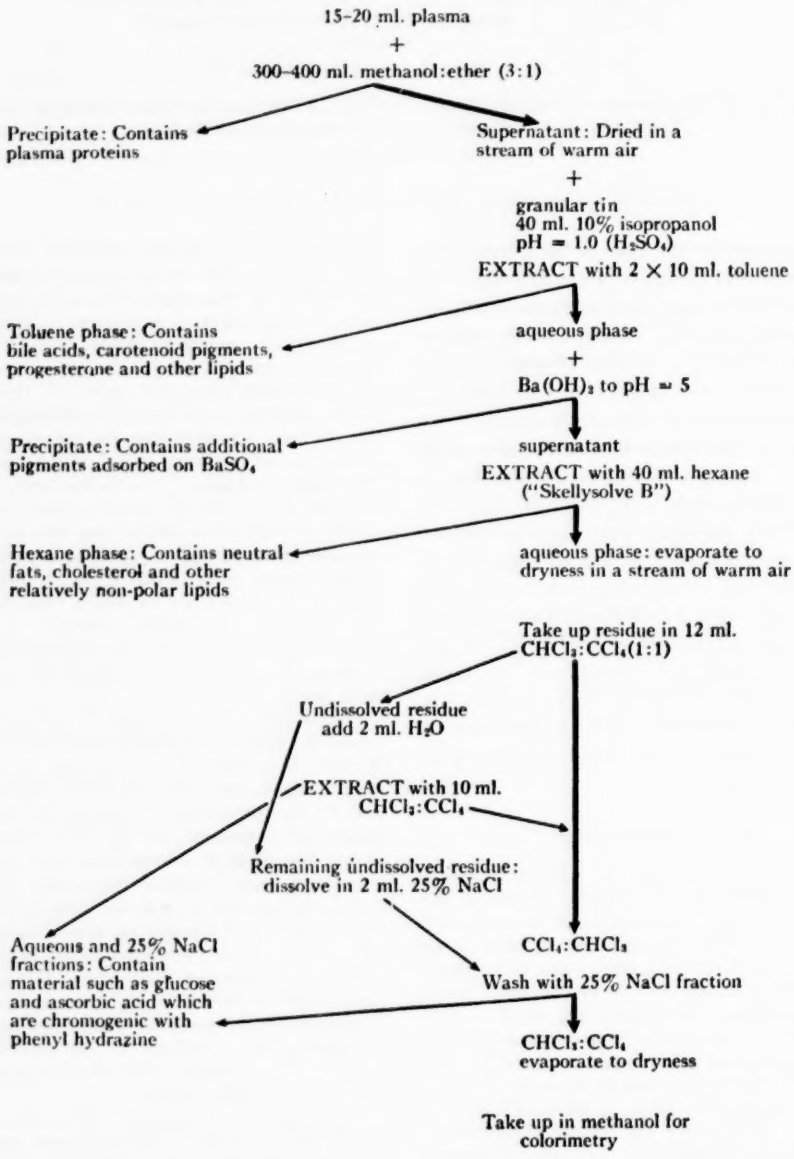
[2] Present address: Department of Internal Medicine, Yale U. School of Medicine, New Haven, Conn.

[3] Kindly supplied by Dr. Dwight Ingle, of the Upjohn Company, Kalamazoo, Mich.

TABLE I

Schematic flow diagram of method of purifying plasma extracts preparatory to applying the Porter-Silber reaction


15–20 ml. plasma
+
300–400 ml. methanol:ether (3:1)
Precipitate: Contains plasma proteins
Supernatant: Dried in a stream of warm air
+
granular tin
40 ml. 10% isopropanol
pH = 1.0 (H_2SO_4)
EXTRACT with 2 × 10 ml. toluene
Toluene phase: Contains bile acids, carotenoid pigments, progesterone and other lipids
aqueous phase
+
$Ba(OH)_2$ to pH ≈ 5
Precipitate: Contains additional pigments adsorbed on $BaSO_4$
supernatant
EXTRACT with 40 ml. hexane ("Skellysolve B")
Hexane phase: Contains neutral fats, cholesterol and other relatively non-polar lipids
aqueous phase: evaporate to dryness in a stream of warm air
Take up residue in 12 ml. $CHCl_3$:CCl_4(1:1)
Undissolved residue add 2 ml. H_2O
EXTRACT with 10 ml. $CHCl_3$:CCl_4
Remaining undissolved residue: dissolve in 2 ml. 25% NaCl
Aqueous and 25% NaCl fractions: Contain material such as glucose and ascorbic acid which are chromogenic with phenyl hydrazine
CCl_4:$CHCl_3$
Wash with 25% NaCl fraction
$CHCl_3$:CCl_4 evaporate to dryness
Take up in methanol for colorimetry

toluene. It is inadvisable to shake this mixture too violently, because stable emulsions may form. The toluene-isopropyl alcohol mixture is transferred into the centrifuge tubes. The residue of the plasma extract remaining in the bottom of the two-liter flask is washed twice more with 10 ml. portions of 10 per cent isopropyl alcohol, which are added to the centrifuge tubes. The sample is centrifuged at 3000 r.p.m. for 15 minutes, after which the bottom alcohol layer is removed to other centrifuge tubes with a 30 ml. syringe equipped with a 4 inch No. 18 needle.

In this extraction, the acid pH makes bile acids relatively more soluble in the lipid phase than would be the case at neutral pH, and they are transferred to the tolu-

ene. The same effect probably also occurs with the phenolic steroids such as the estrogens. The toluene phase becomes brilliantly colored with yellow pigments, presumably carotenoids.

It was found that only about 50 per cent of the hydrocortisone could be recovered when the isopropanol: toluene partition was carried out at pH 1. The lost steroid could not be found in either phase, and was, therefore, presumably destroyed in the acid medium. The destructive effect of the low pH could be completely prevented by the addition of granular tin, as described above.

Using pHydrion paper, the pH is adjusted to 5 to 6 with a saturated solution of barium hydroxide (about 2 ml.). The resulting precipitate of barium sulfate, with small amounts of adsorbed lipids, is removed by centrifugation at moderate speed for 15 minutes. The supernatant is shaken with an equal volume of hexane ("Skellysolve B") to complete the removal of non-polar lipids. The agitation should not be too vigorous, since excessive shaking may produce a stable emulsion. The aqueous phase, which contains the corticosteroids and water-soluble materials, is evaporated to dryness in a 125 ml. Erlenmeyer flask under a stream of air at a temperature of 55° C., with precautions to assure adequate, but not prolonged, drying.

It was found that simple extraction of the dry residue with CCl_4 failed to dissolve all of the hydrocortisone, probably because of mechanical trapping of the steroid. It was therefore necessary to dissolve the lipid-insoluble material in water in order to permit adequate extraction with the lipid solvent. This is accomplished as follows:

The flask is rinsed with four 3 ml. portions of CCl_4, which are pooled in the colorimetry tube. The remaining residue is taken up in 2 ml. of distilled water and shaken against 10 ml. of equal parts of chloroform and carbon tetrachloride. The evaporation flask is rinsed with a 2 ml. portion of 25 per cent NaCl which is put into another separatory funnel and used to back-extract the above $CHCl_3$–CCl_4. All the CCl_4 and $CHCl_3$–CCl_4 fractions are pooled in the colorimetry tube and evaporated to dryness, observing the above mentioned precautions. During all manipulations, the sample must be carefully protected against contact with rubber. All solvents are redistilled shortly before using. The air stream used for blowing off the solvents is filtered through a double train of absorbent cotton.

Colorimetry is carried out by adding 0.8 ml. of absolute methanol and 1.2 ml. of the acid-hydrazine reagent (1.6 mg. of recrystallized phenyl hydrazine per ml. of 20 N H_2SO_4). The tubes are cooled as the reagent is added to prevent heating when the methanol and sulfuric acid are mixed. Readings were made using a Coleman Jr. spectrophotometer with an 18 mm. × 125 mm. tube holder modified to reduce the path of light to a slit 8 mm. wide, with an 18 mm. light path. Optical densities are read at 400 and 600 mμ exactly five minutes after the addition of the reagents. The sample is then incubated for one hour at 60° C., cooled rapidly, and re-read at 400 mμ and 600 mμ. The time required for the color to reach a maximum depends on the temperature at which the reaction proceeds. In the original method of Porter and Silber, the color development was carried out at 80° C. for 20 minutes. We found it more convenient to develop the color for 60 minutes at 60° C.

Calculations: The reading at 400 mμ before heating corrects for nonspecific color produced by the sulfuric acid in the reagent; and the readings at 600 mμ correct for minimal turbidity which may occasionally occur. The corrected optical density is calculated as follows:

$$OD_c = OD_{400} - (OD'_{400} + OD_{600} - OD'_{600})$$

where

OD_c = corrected optical density,
OD_{400} = optical density observed at 400 mμ after 60 minutes heating,
OD'_{400} = optical density observed at 400 mμ before heating,
OD_{600} = optical density at 600 mμ after 60 minutes heating,
OD'_{600} = optical density at 600 mμ before heating.

The resulting value of OD_c is read from a standard curve for Compound F.[4] All solutions are read against a methanol-phenyl hydrazine blank.

The calculation of OD_c by this method fails to take into account nonspecific color which may develop during the incubation of sulfuric acid with the plasma extract. The Porter-Silber method may be modified by splitting the reaction into two steps. The colorless phenylhydrazone can first be formed at pH = 1.0, and, as a second step, the concentration of H_2SO_4 can be brought to 12 N and the color read immediately. When this is done, the extract need not be incubated at elevated temperatures with high concentrations of H_2SO_4, and the nonspecific color formed by sulfuric acid appears at once if it is to appear at all. Since the intensity of the color formed by the one-stage reaction was identical with that produced by the two-stage methods, it was concluded that no increased nonspecific color appears as a result of exposing the plasma to hot H_2SO_4, and no correction was included for this possible source of error.

By this method, it is possible to estimate the plasma concentration of neutral lipids giving a color with a peak absorption at 400 mμ with acid phenyl hydrazine (Figure 1). Since the reaction is quite specific for steroids with a dihydroxyacetone group on the 17-carbon, the color may be considered as a result of the presence of 17-hydroxycorticosterone (Compound F), 17-hydroxy-11-dehydrocorticosterone (Compound E or cortisone) and 17-hydroxy-11-desoxycorticosterone (Compound S). Of these, only Compound F is believed to be present in significant quantities in normal human plasma (4). The extraction procedure completely removes progesterone (which causes a small amount of color at 410 mμ), and all of the water-soluble materials such as ascorbic acid which are strongly chromogenic in the Porter-Silber method. The method will not detect adrenal cortical steroids which have no 17-hydroxyl, and therefore it will not measure corticosterone, which may represent an appreciable fraction of

[4] Generously supplied by the Merck Company.

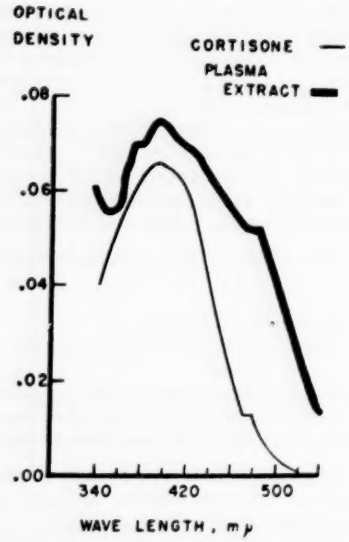


FIG. 1. ABSORPTION CURVES OF THE PHENYL-HYDRAZONE OF PLASMA EXTRACT COMPARED WITH THAT OF 3 μGM. OF CRYSTALLINE CORTISONE

the steroid secretion of the human adrenal gland. Since, however, it is estimated that Compound F is the chief adrenal steroid present in the plasma of experimental animals (5) and since Compound F is more potent than corticosterone in promoting glycogen deposition (6) in protecting against adrenalectomy (7), and in maintaining muscular strength (8), it was felt that measurement of Compound F would give an estimate of a large part of the adrenal steroid activity in the plasma.

This method yields recoveries of 85 to 112 per cent of adrenal cortex extract added to plasma, and 60 to 70 per cent of hydrocortisone acetate is recovered after addition to plasma. The standard error of the difference of duplicate analyses equals 5.1 per cent, indicating that a difference of 15.5 per cent between means can be detected with $p = .05$.

Evidence that the Material Measured is Related to Adrenal Cortical Function. Although it seemed theoretically probable that the chromogen measured by the method described is in fact an adrenal steroid, it was desirable to demonstrate that the material being studied actually originates in the adrenal gland. For this purpose three types of evidence were sought.

In human beings with adrenal cortical insufficiency, the concentration of the chromogen is lower than in normal human beings (Table II). The fact that it is not entirely absent may mean that the analytical method is not totally specific, and that small amounts of nonspecific (i.e., nonadrenal) chromogenic material are included in the assay. It is also possible that, in some patients with clinical adrenal insufficiency, small amounts of adrenal cortical hormones may still be secreted and appear in minute amounts in the blood. After severe, acute trauma, the chromogenic material is found in the plasma in increased concentration. The range of normal values (3 to 13 μgm. per 100 ml.) is similar to that found by Nelson, Samuels, Willardson, and Tyler (4) using another method of preparing plasma for application of the Porter-Silber reaction.

After the administration of adrenocorticotrophic hormone (ACTH), the concentration of the chromogen is increased (Table III). The peak concentration of the chromogen may not have been observed, since Nelson, Samuels, Willardson, and Tyler have shown (4), using a different method of analysis of the plasma, that the peak elevation of the material measured by their method occurs somewhat earlier than the four-hour time interval used in this study.

TABLE II

Concentration of 17-hydroxycorticosteroids in the plasma of normal subjects, of patients with adrenal cortical hypofunction, and of patients after severe acute trauma

Normal subjects

Number	Range μg./100 ml.
12	3.0 – 5.9
7	6.0 – 8.9
6	9.0 – 11.9
5	12.0 – 13.7
30	7.8± 3.3 (Mean±St. Dev.)

Hypoadrenal subjects

Disease	Concentration of 17-OH-corticosteroids μg./100 ml.
Hypopituitarism*	2.0
Addison's Disease*	1.2
Addison's Disease*	3.2
Adrenalectomy	2.3

Patients After Severe Acute Trauma

Severe gastrointestinal upset with dehydration and hyponatremia	16.7
Fracture, left femur	16.3
Lobar pneumonia	26.3
Bleeding peptic ulcer	17.7
Acute pyelonephritis	26.7
Myocardial infarct	16.3
Brachial plexus injury	17.7
I.V. Typhoid bacilli for pyrogen therapy	24.3

* These sera were kindly donated by Dr. Gilbert Gordan, University of California Medical School.

An additional important point is the fact that the concentration of the chromogen is higher in adrenal vein blood than in peripheral blood. Adrenal vein blood was obtained by anesthetizing rabbits with pentobarbital intravenously, and tying off in succession the renal pedicles, the vena cava below the kidneys, and the vena cava above the adrenal glands. The segment of inferior vena cava thus isolated received drainage from the adrenal glands and also from several small parietal veins. Blood obtained from this segment does not, therefore, represent pure adrenal vein blood, but a large part of the blood so

obtained does flow from the adrenal glands. The concentration of the chromogen in plasma from a peripheral vein was 4.0 μgm. per 100 ml., whereas that in the isolated inferior vena cava segment was 16.0 μgm. per 100 ml. in one experiment. In a second experiment, the arterial concentration was 27.6 μgm. per 100 ml., whereas adrenal vein plasma contained 188 μgm. per 100 ml.

In view of this evidence, it seems probable that most of the material measured by the method described originates in the adrenal gland, and consists, in fact, of one or more of the 17-hydroxycorticosteroids.

Experimental Design. The catheter was directed, under fluoroscopic control, through the left brachial vein, via the superior vena cava and right atrium, into the inferior vena cava, and thence into the left renal vein. It was necessary to obtain blood from the left renal vein, since it is only on the left side that adrenal blood drains into the renal vein. In the ideal experiment, the catheter was placed in two positions. In one the tip of the catheter was pushed out as far as it would go with ease. This position was distal to the entrance of the adrenal vein, and was intended as a control on the excretion of the adrenal steroids by the kidney (Figure 2, A). The second position was as close as possible to the junction of the left renal vein and the inferior vena cava. This position was proximal to the usual point of entry of the adrenal vein, and made it possible to sample a mixture of renal and adrenal venous blood (Figure 2, B). Throughout the remainder of the paper, these two positions of the catheter tip will be referred to as RVA and RVB, respectively.

After the catheter had been placed deep in the renal vein, a priming dose of 6.7 mg. per kg. of sodium para-amino hippurate (PAH) was given intravenously, after which an infusion of 200 mg. of PAH in 100 ml. of saline was injected into the vein by an accurately controlled electrical pump at a rate of about one ml. per minute. An indwelling arterial needle was inserted, and a period of 30 to 45 minutes was then permitted to elapse so that the PAH concentration in the blood could become stable, and so that the subject could relax after the relatively minor trauma of the initial manipulations. All instrumentation was performed under adequate metycaine local anesthesia, and none of the patients complained of pain at any time during the procedure.

After the 30-minute equilibration period, one ml. of heparin was injected into the catheter. Samples were taken from the artery and catheter for PAH determination before and after each 17-hydroxycorticosteroid sample. Corticosteroid samples were taken from the catheter and, immediately thereafter, from the artery, first with the catheter tip distal to the entrance of the adrenal vein (Position A, Figure 2), and (usually about five minutes later) with the tip proximal to the adrenal vein (Position B, Figure 2). In some instances, 25 units of ACTH were given intravenously after the second corticosteroid sample, and a third set of samples was taken 5 to 10 minutes later.

TABLE III

Effect of adrenocorticotrophin (ACTHAR Armour) on the concentration of plasma 17-hydroxycorticosteroids

	17-hydroxycorticosteroid concentration μg./100 ml. plasma		Interval between injection and 2nd sample
	Control	After ACTH	
1	5.0	17.3	4 hrs.
2	13.0	15.7	4 hrs.
3	4.7	12.7	4 hrs.
4	8.6	10.4	10 min.

Each subject received 25 units of ACTH intravenously immediately after the control sample was drawn.

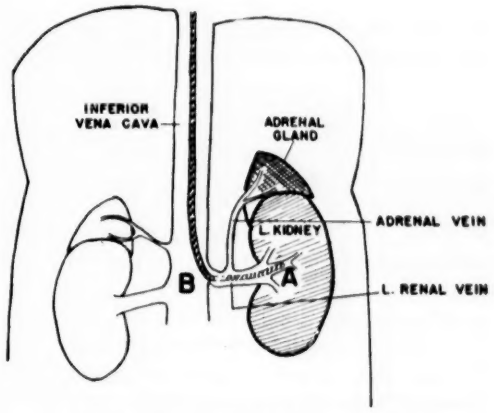


FIG. 2. POSITIONING OF THE CATHETER

At "A," pure renal venous blood is obtained.

At "B," adrenal vein blood is mixed with the venous blood.

Calculations. The renal plasma flow was estimated by the Fick principle, assuming that the only route of exit for PAH from the blood is via the kidneys. This assumption is supported by the fact that the concentration of PAH in samples taken from various veins was indistinguishable from the concentration in the artery. A correction for changing arterial PAH level could have been made by the method suggested for hepatic blood flow estimations by Bradley, Ingelfinger, Bradley, and Curry (9), but arterial PAH levels were sufficiently constant that this correction would have been negligible, and it was therefore ignored. The renal plasma flow was therefore calculated from the formula:

$$RPF = \frac{I}{A - RV}$$

where RPF = renal plasma flow, I = the rate of infusion of PAH in mg. per minute, and A and RV = arterial and renal venous concentrations of PAH in mg. per ml. Since the value for "I" is critical, it was obtained by collecting the outflow from the infusion pump for exactly five minutes at the end of each experiment, analyzing the effluent for PAH, and dividing the result by 5.

The method of estimating renal plasma flow described above actually measures the rate of flow at the tip of the

TABLE IV

Estimation of the output of 17-hydroxycorticosteroids by the human adrenal gland

Name	Age Sex	Wt., kg.	RPF ml./min.	17-Hydroxycorticosteroid concentration μg./100 ml. plasma					Output	
				Art.	RV-A*	Art.	RV-B*	RV-B*-Art.	μg./min.	mg./24 hr.
H. R.	13 M	56.8	762			10.8	12.8	2.0	15.2	22.0
R. C.	36 M	68.8	933			11.3	13.3	2.0	18.7	26.9
J. C.	30 M	67.1	835	6.7	6.9	6.0	6.2	0.2		
J. M. T.	15 M	65.0	865	15.6	15.6	20.0	21.4	1.4	12.1	17.4
D. P.	41 M	59.6	707			8.6	11.4	2.8	19.8	28.5
				After ACTH 17-OH-corticosteroid concentration						
J. M. T.	15 M	65.0	935	15.3			21.3	6.0	56.1	80.8
D. P.	41 M	59.6	1257	10.4			12.8	2.4	30.2	43.5

* RV-A and RV-B refer to positions of the catheter shown in Figure 2.

catheter, since dilution of pure renal venous blood by contaminating (e.g., adrenal, ovarian or spermatic vein) blood increases the concentration of PAH in RV. This will decrease the value of A–RV, and increase the estimated plasma flow.

The adrenal corticosteroid output was estimated by subtracting the arterial 17-hydroxycorticosteroid concentration from the renal venous concentration, and multiplying the result by the renal plasma flow. The assumptions implicit in this calculation will be discussed below.

RESULTS

The results obtained in five experiments are shown in Table IV. In each case except one, the concentration of 17-hydroxycorticosteroids was higher in the RV-B sample than in the corresponding arterial sample. Two possible explanations can be suggested for the absence of a difference in J. C. It is possible that the catheter tip was improperly placed, so that sample "RV-B" was actually taken distal to the point of entry of the adrenal vein. It is also possible that this patient had an anomalous adrenal vein which entered the vena cava instead of the left renal vein.

It is noteworthy that the arterial concentration of plasma 17-hydroxycorticosteroids was not elevated above normal levels in four of the five subjects. After the injection of ACTH, the estimated adrenal corticosteroid output was appreciably increased in the two subjects in whom it was possible to perform this experiment. In one case, however (D. P.), the increase was entirely a result of increased renal plasma flow.

DISCUSSION

Several fundamental assumptions must be made before the data presented above can be accepted at face value. The most important of these is that adrenal venous blood is uniformly mixed with renal venous blood by the time it reaches the catheter tip. It is highly unlikely that this occurs, since the flow of rapidly moving blood masses tends to stream in discrete layers. The proportions of admixture of the renal and adrenal blood will depend entirely upon the position of the orifice of the catheter tip in relation to the margins of the two streams. Since, however, the renal blood flow is very rapid, and since the presence of the catheter itself probably produces some turbulence, a considerable, unpredictable degree of mixing may occur. To some extent, therefore, the results obtained by the catheter will be determined by chance.

The catheter technique samples a mixture of blood from the left adrenal and kidney. The method of calculation of renal plasma flow assumes that left and right kidneys are equally active and that similar PAH extractions would be obtained if both renal veins were samples. The calculated RPF is, therefore, the total plasma flow for both kidneys. Since the actual observations are made

only on the left side, it must be assumed that the activity of left and right adrenals is equal. The method of calculation, in fact, results in a value for double the output of the left adrenal, rather than the combined output of right and left adrenal glands. While it is probably correct to assume that the activity of the two glands is usually equal, this is not known in any given case, and grossly incorrect results would be obtained if either gland were very much more active than the other.

The volume of blood flowing through the kidney is so large that the volume of adrenal venous blood added is not significant in disturbing the calculation of renal plasma flow. In most cases, more than 95 per cent of the PAH was removed from the blood obtained through the catheter, indicating that more than 95 per cent of the blood had been exposed to the excretory activity of the renal tubules. This fact results in a very great dilution of the adrenal venous blood. Consequently, the differences in the concentration of 17-hydroxycorticosteroids in arterial and renal vein blood are small. In order to calculate the corticosteroid output, this small difference must be multiplied by the very large renal plasma flow. As a result the importance of analytical inaccuracies in the determination of the adrenal steroids is exaggerated many fold. The calculated output of adrenal steroids must, therefore, be accepted only with grave reservations.

In view of the serious criticisms of the experimental approach which have just been mentioned, it is interesting to find that the results obtained are actually reasonable and consistent with previous observations by other methods. The "resting" output of the human adrenal gland in the four experiments which were successful was equivalent to 17 to 29 mg. of Compound F equivalents per day. These values are not very different from the amounts of cortisone (15 to 25 mg. per day) found suitable for maintenance therapy in patients with adrenal cortical insufficiency (10). The quantities of steroid secreted five minutes after ACTH approximate the dose of cortisone which has been used for the treatment of patients with Addison's disease subjected to operative trauma (100 mg. per day or so). These results also suggest that the administration of ACTH can result in the secretion of quantities of 17-hydroxycorticosteroids comparable to the therapeutic doses of cortisone used in the treatment of arthritis and other inflammatory diseases.

SUMMARY

A new method is described for estimating the concentration of 17-hydroxycorticosteroids in plasma. The material measured is of adrenal origin, as evidenced by the fact that it is reduced in patients with Addison's disease, increased after the administration of adrenocorticotrophic hormone, and increased in plasma from the adrenal vein as compared with arterial plasma. The normal concentration range is 3 to 13 μgm. per 100 ml. plasma. The rate of secretion of 17-hydroxycorticosteroids has been estimated in normal human subjects by collecting simultaneous samples of left renal vein and arterial blood, and determining the difference in concentration of the hormone. The rate of release of the hormones can be estimated by multiplying this difference by the renal plasma flow. In the experiments described, the hormone was secreted at a rate equivalent to 15 to 25 mg. of hydrocortisone per day. The limitations of the method are discussed at length. In view of the inherent inaccuracies of the technique, other methods of evaluating the effect of various agents on adrenal secretion must be found.

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CINNAMIC ACID AS A TEST SUBSTANCE IN THE EVALUATION OF LIVER FUNCTION [1]

By ABRAHAM SALTZMAN AND WENDELL T. CARAWAY

(From the Medical and Laboratory Services of the Rhode Island Hospital, Providence, Rhode Island)

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INTRODUCTION

Previous quantitative studies of the urinary metabolites of cinnamic acid in human subjects have given promise of yielding data of clinical value in hepatic disease (1, 2). As is well known from both animal and human experiments, this relatively non-toxic substance is converted to and excreted as hippuric acid in the urine. The possibilities of this test material as a model for the study of the rate of beta-oxidation by the liver of the intact subject were first suggested by Snapper (3). Snapper, Yü and Chiang demonstrated that, in the human subject, the portion of cinnamate which escaped oxidation was excreted by conjugation as cinnamoylglucuronic acid (4). Furthermore, in their subjects free cinnamic acid and cinnamoylglycine were not detected.

After oral administration of 6.9 grams of sodium cinnamate, Snapper and Saltzman showed (2) that normal subjects oxidize the main part of the cinnamate to benzoate, the remainder being conjugated as a glucuronide. In liver disease the rate of oxidation of cinnamate was decreased, and when the benzoate was produced more rapidly than it could be removed by an impaired glycine conjugation mechanism, benzoylglucuronide was formed. It was also found that by giving comparable amounts of the more insoluble and slowly absorbed cinnamic acid rather than its salt, there was a more complete oxidation of cinnamate to benzoate and less glucuronide excretion in the urine.

It would appear from the above that the preferred metabolic pathway is beta-oxidation to benzoate, with a secondary mechanism of removal as cinnamoylglucuronic acid. That the latter "defense" mechanism can be brought into greater use with impairment of oxidative function was seen in hepatic disturbances where a uniform dose of 3 grams of cinnamic acid was given to all subjects. Upon administration of the latter, a semi-quantitative test for excessive excretion of glucuronides was performed on fractional urine specimens. In normal subjects there was little or no reaction with the naphthoresorcinol reagent while in hepatocellular disease almost all subjects tested showed increased excretion of glucuronides, often with a lag period. By comparison with the benzoylglucuronic acid test, greater sensitivity was noted with cinnamic acid as the test substance. The lack of sensitivity of the oral benzoic acid test has also been noted by Sharnoff, Budnick and Jakab (5).

With the development by the authors of a method of estimation of serum cinnamic acid, a further clinical trial of cinnamic acid as a test material in the evaluation of liver function in various disease states appeared warranted. The intravenous route of administration with sodium cinnamate was chosen to overcome the marked variation in rates of intestinal absorption of cinnamic acid. It will be demonstrated that the rate of removal of cinnamate from serum, which largely is an index of hepatic oxidative ability, was closely correlated to the extent of liver injury as determined by the clinical progress of the patient and other tests.

METHODS

Clinical Procedure:

1. *Oral test.* Patient is fasting and receives neither medications nor i.v. glucose. A control specimen of blood is obtained. Cinnamic acid is given orally in gelatin capsules 0.6 gram per 30 lb. with water. Repeat blood specimens are taken hourly for a two hour period and an entire six hour urine sample saved for hippuric acid determination.

2. *Intravenous test.* Patient is fasting as above. After a control specimen is drawn, the syringe is detached and through the same needle sodium cinnamate is in-

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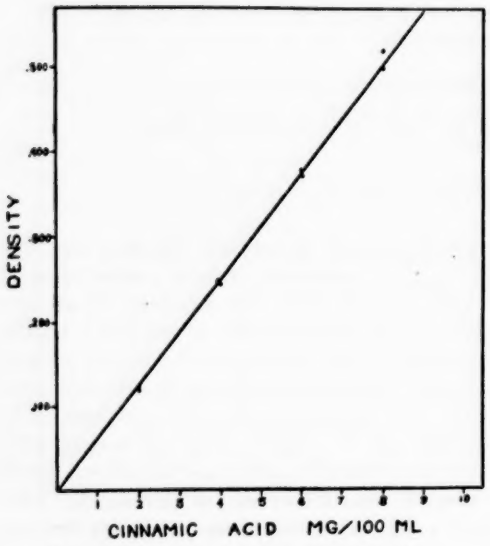

FIG. 1. STANDARD CURVE OF CINNAMIC ACID
Optical density of sodium cinnamate added to serum, blank subtracted, with measurement at 270 mμ in the Beckman Spectrophotometer.

jected in dosage of 5 mg. per kg.[2] Repeat specimens of blood are taken at timed intervals, 5 and 20 minutes.

Analytical Methods:

Blood serum was used in all tests. Glucose was determined by the Benedict method (6) and inorganic phosphorus by the method of Benedict and Theis (7). Serum glucuronic acid was determined by a modification of the naphthoresorcinol reaction as described by Fishman, Smith, Thompson, Bonner, Kasdon, and Homburger adapted to blood serum (8). The standard curve was prepared with glucuronolactone. Readings were made at 580 mμ (rather than the described 565 mμ) where the peak absorption with the Beckman apparatus was obtained. An essential element in obtaining reproducible data with this procedure is the scrupulous care given to the glassware. An important step is to leave the boiling tubes in 5 per cent KOH overnight. The readings obtained from sera of patients given invert sugar intravenously the previous day will be increased due to the interference of a brown color extracted by toluene.

An ultraviolet absorption procedure was devised for the estimation of cinnamic acid in serum. Sodium cinnamate added to the precipitating cadmium hydroxide reagent showed a strong peak of absorption at 270 mμ at which wavelengths benzoic, hippuric and phenylpropionic acids have almost no absorption. Cadmium hydroxide removes bilirubin as well as many constituents of serum absorbing in the ultraviolet without appreciable loss of cinnamic acid. Recovery experiments show that 90 to 98 per cent of the cinnamate is retained in the filtrate. A serum blank before the test is always necessary. This is a critical feature of the method, significant variations in the blank being present in some samples. Sulfonamides in particular may elevate the serum blank.

To duplicate 0.5 ml. portions of serum, add 0.5 ml. of water, 5.0 ml. of 0.58 per cent cadmium sulfate solution and 1.0 ml. of 0.1 N NaOH. Place in boiling water bath for four minutes, cool, filter. An additional 2 ml. of water is used to complete the transfer. For the reagent blank, water is used in place of serum. The filtrate is then placed in a 1 cm. quartz cell and measured in the Beckman Spectrophotometer at 270 mμ. Duplicate analyses should agree within 5 per cent.

The stock standard solution is sodium cinnamate C.P., 1.15 grams per liter. It is diluted ten-fold to give concentration equivalent to 100 micrograms of cinnamic acid per ml. The standard curve (Figure 1) is obtained by

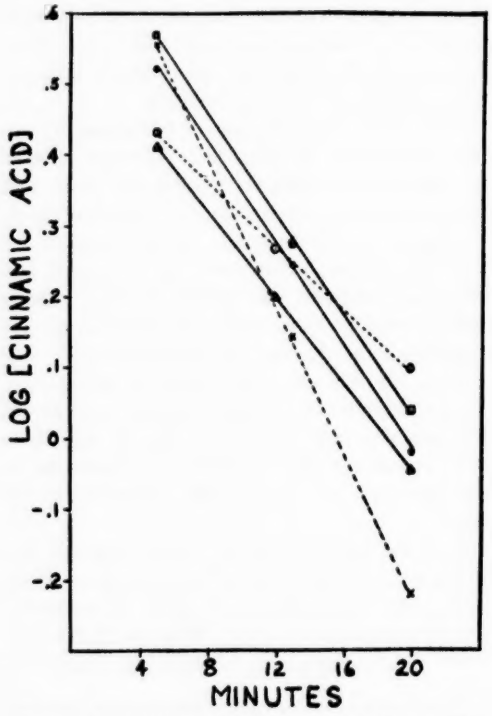

FIG. 2. LOGARITHMIC DISAPPEARANCE OF SODIUM CINNAMATE FOLLOWING INTRAVENOUS INJECTION (5 MG. PER KG.)

[2] The Eli Lilly Research Laboratories, Indianapolis 6, Ind. kindly prepared a highly purified, sterile, aqueous solution of sodium cinnamate for intravenous usage. A 5 per cent solution was employed, but as that concentration is near the saturation limit, a 2.28 per cent solution was later prepared for stability. The latter is approximately isotonic, and dosage is 0.1 cc. per 10 lb. body weight.

addition of 0.5 ml. of standard cinnamate in varying concentrations to 0.5 ml. of pooled serum and proceeding with the analysis as described above. A serum blank, containing no added cinnamate, is subtracted and the resultant optical density plotted against equivalent serum cinnamic acid. A straight line relationship is obtained, which for our Beckman Model DU Ultraviolet Spectrophotometer followed the equation:

(density unknown—density blank) × 15.8 = mg. cinnamic acid per 100 ml. serum.

This factor (15.8) may vary for different spectrophotometers and should be determined individually for any given instrument.

To indicate the precision of the method, in a series of 100 consecutive analyses, the duplicate determinations differed by a mean of 0.10 mg. per 100 ml. serum (S.D. = 0.08). In addition it was thought worthwhile to determine the lowest level of cinnamic acid concentration which could be determined with accuracy by the ultraviolet technique in order to establish the significance of cinnamic acid levels reported in the lower range (0.3 to 2.0 mg. per 100 ml.). Recovery experiments with concentrations at intervals in this range indicated an accuracy within 0.1 mg. per 100 ml., sufficient for our purposes.

Following intravenous administration the cinnamate disappears rapidly from the blood. By measuring the concentration of serum cinnamate at intervals in five subjects, it was found that the decrease in concentration with time was logarithmic, *i.e.*, followed a first order reaction as shown in Figure 2. The rate of decline of cinnamate level is equal to the value for the slope of the straight line and is expressed as per cent change in concentration per minute:

$$\frac{\Delta \log C}{\Delta t} \times 100 = K$$

The hippuric acid in urine was determined by the gravimetric procedure of Quick with the addition of ammonium sulfate to decrease the solubility of the hippuric acid in the filtrate (9). Results are expressed as per cent conversion of cinnamic to hippuric acids in the six hour interval.

OBSERVATIONS

The data obtained with the oral cinnamic acid test are shown in Figure 3. The peak serum cinnamic acid level was reached in either the 1st or 2nd hour specimen in 19 out of 20 subjects studied hourly for four hours after cinnamic acid ingestion. Because of the variation shown in the rate of intestinal absorption, the mean 1 and 2 hour level was taken for comparative purposes. In 23 normal subjects the serum cinnamic acid level was in

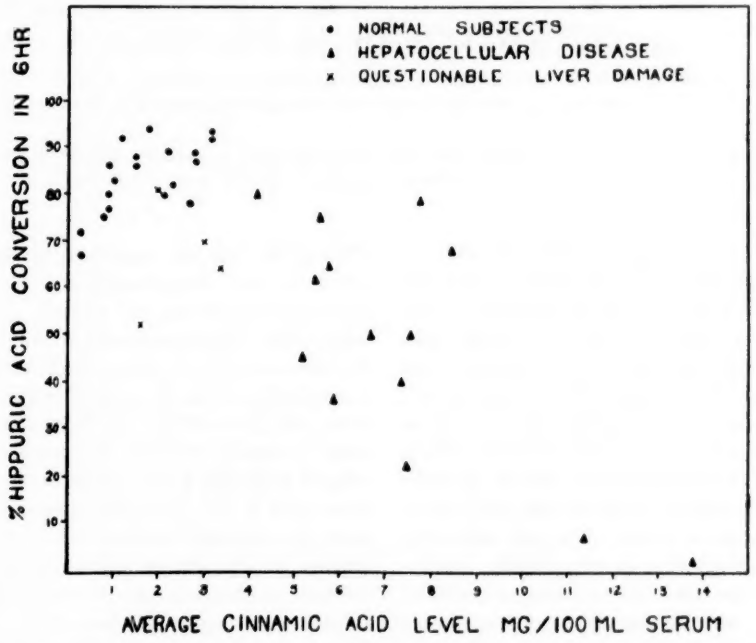

FIG. 3. AVERAGE OF THE 1 AND 2 HOUR SERUM CINNAMIC ACID LEVELS WITH RELATION TO PER CENT CONVERSION OF CINNAMIC TO HIPPURIC ACIDS IN A SIX HOUR PERIOD IN NORMAL SUBJECTS AND PATIENTS WITH HEPATOCELLULAR DISEASE

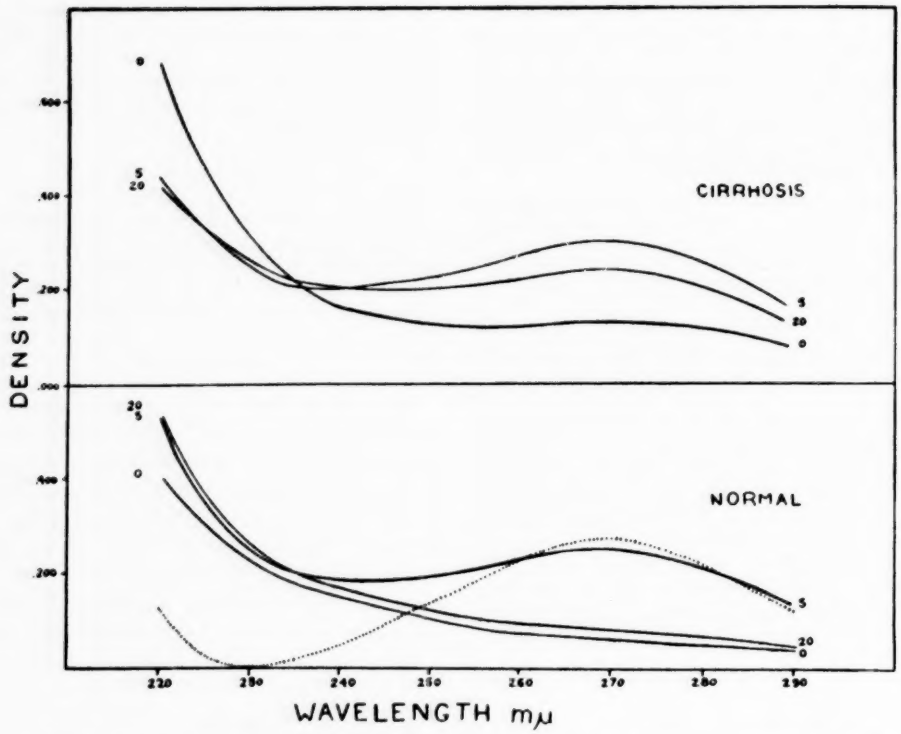

FIG. 4. ULTRAVIOLET ABSORPTION CURVES OF SERUM FILTRATES OF A NORMAL SUBJECT AND A PATIENT WITH PORTAL CIRRHOSIS AS PREPARED WITH THE CADMIUM HYDROXIDE PRECIPITATION METHOD OF TEXT

Blood was drawn at 0, 5, and 20 min. after intravenous injection of sodium cinnamate in dosage of 5 mg. per kg. The absorption curve of sodium cinnamate in distilled water is indicated by the dotted line.

the range of 0.3 to 3.4 mg. per 100 ml. with absorption and conversion of cinnamic to hippuric acids of 67 to 94 per cent of the theoretical yield in the six hour period. The 17 patients with hepatocellular disease had serum cinnamic acid levels of 4.2 to 13.8 mg. per 100 ml. and showed decreased conversion to hippuric acid as a group although there was some overlap with the normal controls. There was no decided change in either the glucose, phosphorus or glucuronic acid values in any of the subjects tested. The only side effect was nausea and vomiting in one patient.

In Figure 4 the ultraviolet absorption curves of serum at 0, 5, and 20 minutes after intravenous injection of sodium cinnamate 5 mg. per kg. are given in two typical instances. In the 5 minute specimen the normal subjects had levels of 2.7 to 4.9 mg. per 100 ml. expressed as cinnamic acid, while in liver disturbances the 5 minute level ranged 1.9 to 5.0 mg. per 100 ml. In the normal subject the 5 minute specimen shows the cinnamate maximum at 270 mμ with a rapid decline to almost the control level in the next 15 minutes. The patient with liver damage, as illustrated by a patient with Laennec's cirrhosis, has a somewhat higher control level and a less marked fall of the cinnamate peak in the 20 minute specimen. Furthermore, in one-third of the cases of hepatocellular damage the observation was made that, as in this instance, both the 5 and 20 minute levels at the shorter wavelengths fall below the control level. The explanation for the latter is not known.

To date 125 intravenous sodium cinnamate tests have been performed. Side effects are a transient

flushing in about one-fifth of the patients and in an occasional patient an epigastric cramp-like pain several minutes in duration. These reactions do not correlate to the cinnamate level attained, nor are they a constant finding with an individual patient in whom the procedure is repeated. There was no febrile response.

In Figure 5 the distribution of rates of decline of serum cinnamate in 105 tests in normals and in various pathologic conditions [3] are given. The range for 18 carefully examined normal subjects, aged 14 to 64 years, was 4.0 to 6.0 per cent per minute. In the group with cirrhosis of the liver, the majority of values was between 0.8 and 3.1 per cent per minute with several higher values obtained in patients in a latent phase of their illness. Serial determinations showed a direct correlation of increased rates of cinnamate removal with improvement in the clinical status and remarkably constant rates in the unimproved patient. Weekly determinations of K in a patient with Laennec's cirrhosis undergoing a remission, for example, gave the following results: 1.8, 2.0, 2.3, and 2.7. In hepatitis the initial values are usually low in the acute phase of the illness, rising with clinical improvement until values within the normal range are obtained. The congested liver due to heart failure may obtain a low value during the acute episode with rapid improvement as the cardiac condition is brought under control. For example, one such patient had a value of 1.6 on admission which rose to 2.8 within ten days as the heart failure subsided. The metastatic carcinoma patients, all with obvious clinical involvement of the liver, obtained intermediate values from 2.1 to 3.3 per cent per minute. All six patients with common duct obstruction were explored surgically. Diminished rates of cinnamate removal were found in the three with long-standing obstruction. Slightly diminished rates were present in the post-operative group (2.9 to 3.8). These were patients in the early post-operative stage where there were the factors of nutritional impairment and infection which may have had a bearing. In addition to routine liver function tests, explora-

[3] In the 20 tests not graphed there were miscellaneous diagnoses such as Hodgkin's, leukemia and myeloma which did not fall under the listed headings.

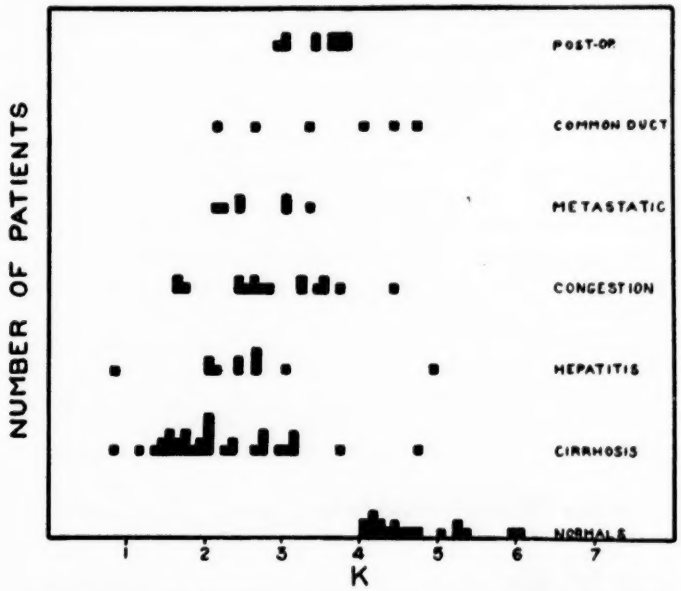

FIG. 5. RATE OF DECLINE OF SERUM CINNAMATE (K) AS PER CENT PER MINUTE CHANGE IN SERUM CONCENTRATION FOLLOWING INTRAVENOUS INJECTION OF SODIUM CINNAMATE IN 105 TESTS IN NORMALS AND VARIOUS HEPATIC AND OTHER DISEASE STATES

TABLE I

*Fasting serum glucuronic acid level mg./100 ml.**

Diagnosis	Weeks in hospital						Comment
	1	2	3	4	5	6	
1. Hepatitis	4.7	2.9					Recovery
2. Hepatitis	7.3	3.6	3.5	3.2			Recovery
3. Hepatitis	4.7	—	4.1	3.1			Recovery
4. Hepatitis	7.7	3.8	3.5	3.5	3.5		Recovery
5. Cirrhosis	6.3	5.0	4.3	4.0			Improved
6. Cirrhosis	6.0	6.4	8.4	4.9	3.7	3.9	Delayed improvement
7. Cirrhosis	4.9	3.6					Improved
8. Cirrhosis	4.3	—	3.6	—	5.9		Unchanged
9. Cirrhosis	4.3	—	3.6				Slight improvement
10. Cirrhosis	5.6	5.5	6.1	5.2			Died in 5th week

* Uncorrected for glucose (see text).

tory operation, autopsy findings, or liver biopsy were utilized in approximately one-third of the patients in arriving at a definitive diagnosis.

In two patients with multiple myeloma, K values over 6.0 per cent per minute were observed (7.9 and 8.0). The latter, in addition, had 5 minute cinnamate levels of only 1.2 and 1.4 mg. per 100 ml., which was considerably lower than any other subject tested and indicative of an accelerated removal of cinnamate. A return of the 5 minute and K values to normal was observed in one patient while under treatment with urethane and cortisone. A third patient with myeloma had a low 5 minute level (1.7) and low K value (3.4) both of which rose to normal (2.0, 4.3, respectively) under urethane therapy. A fourth patient in an early clinical stage of this disease without roentgen evidence of bony destruction had normal findings.

After intravenous cinnamate there was no marked change in the inorganic phosphorus or glucuronic acid levels as compared to the control fasting state. However, an elevation above the normal was seen in the fasting control glucuronic acid value in the patients with active liver damage, the glucose level being unchanged. Thus, with the method given we found that in the normal subject glucuronic acid values (uncorrected for glucose) were in the range 2.3 to 4.0 mg. per 100 ml. (42 determinations with the mean of 3.2 and S.D. of 0.5), while a group of 75 liver patients in various stages of hepatitis, cirrhosis, metastasis and congestion had a mean level of 4.8 mg. per 100 ml. with S.D. of 1.4 (Figure 6). This is a statistically significant difference. In this regard it is of interest that in ten unselected hepatitis or cirrhosis patients in whom repeat determinations were made, elevated values returned to normal in the patients undergoing remission and were unchanged in the unimproved (Table I). With the glucuronic acid method of Fishman, Smith, Thompson, Bonner, Kasdon, and Homburger, a correction for glucose content was not found significant in the normal or liver damage group, but of importance in the diabetic where the deviation due to glucose may be large (10).

DISCUSSION

A summary of recorded observations of metabolic pathways in the fate of cinnamic acid after ingestion by human subjects is indicated in Figure 7. These data are based on measurements of conjugation products excreted in the urine. In the dosage given the normal pathway (counterclockwise in Figure 7) is largely by beta oxidation to benzoic acid and conjugation of the latter with glycine to form hippuric acid. In liver damage there is diminished oxidation to benzoate and diminished glycine production and conjugation, with greater use of the glucuronic acid conjugation system (clockwise in Figure 7).

With the development by the authors of a method of measurement of blood cinnamate level, a further trial of cinnamic acid as a liver test substance was performed. In the liver damage group, the orally administered test demonstrated again the impaired oxidation of cinnamate to benzoate by the elevation of serum cinnamate levels and

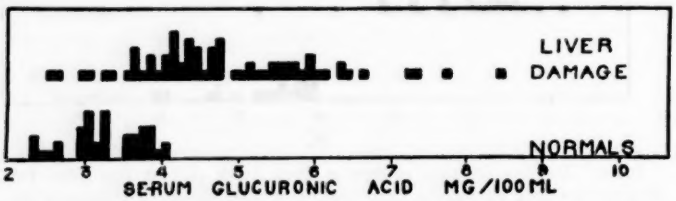

FIG. 6. RANGE OF GLUCURONIC ACID VALUES IN THE FASTING STATE IN NORMAL SUBJECTS AND IN PATIENTS WITH LIVER DISEASE

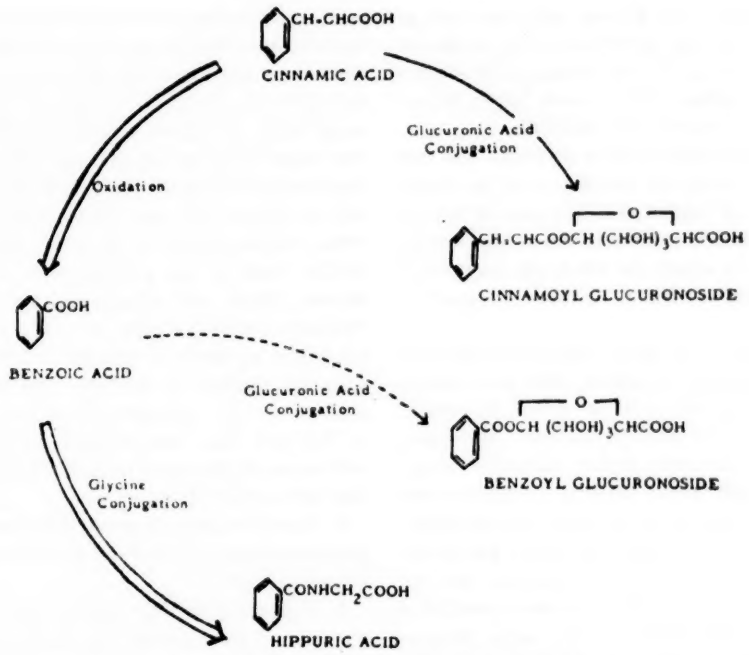

FIG. 7. METABOLIC PATHWAYS OF CINNAMIC ACID

The normal pathway is largely counterclockwise. In liver damage with impairment of oxidation of cinnamate to benzoate and glycine conjugation to hippurate, there is increased demand on the glucuronic acid conjugation system (clockwise).

concomitant decreased excretion of hippuric acid in the urine. Since the glucuronic acid level was unchanged over the control value, it can be assumed that the excess of unoxidized cinnamate was circulating as a sodium salt rather than as a conjugated compound. No significant change was noted in the glucose and phosphorus levels in the patients studied. The latter indicated that the intact human being given the dosage employed in this study reacts differently from isolated rat and guinea pig kidney and liver slices as studied by Weinbach, Lowe, Frisell, and Hellerman (11). They noted that the presence of cinnamate will presumably increase the rate of utilization of glucose as seen from lactate accumulation which is paralleled by increased glucose removal.

The rate of decline of serum cinnamate concentration following intravenous administration appears to offer a suitable index of the oxidative function of the liver. From the distribution of results obtained in various disease states, and from serial determinations made in patients with liver damage with correlation to the clinical status, it is apparent that there is a direct relationship between the functional adequacy of the liver and the rate of removal of cinnamate. The clinical material clearly points to the liver as the principal site of this metabolic activity.

Unlike the sulphobromphthalein sodium retention test, the presence of jaundice interferes neither with the metabolic pathway nor the method of determination of cinnamate. Low rates of serum cinnamate removal were seen in hepatitis, cirrhosis and congestive hepatomegaly in direct relationship with the clinical status of the patient. With improvement in hepatitis the values returned to the normal range, but in the cirrhotics the improvement was not as marked. Normal or near normal values were found in the latent or well compensated cirrhosis patients who were under hospital surveillance for other reasons. Since a close correlation was found between the rate of cinnamate decline in the serum and the severity of the liver injury, the results of this test may have a prog-

nostic importance. No definite statement can as yet be made as to the significance of a particular range of values with this test compared to routine liver function studies until a much larger homogeneous series of patients are studied.

In two patients with multiple myeloma, the rate of removal of cinnamate was increased to almost twice normal. It follows that this may be due, in part, to the metabolic activity of the myeloma cells. In one patient in whom the latter was suppressed by urethane and cortisone, K values dropped to normal.

A mild elevation in the fasting glucuronic acid levels in a proportion of patients with liver disease was noted, along with a rapid return to normal early in the course of improvement. If further observations bear out this finding, it may be a prognostic guide with these patients. Simultaneous glucose determinations were in the normal range. While large doses of drugs may cause glucuronic acid elevation, most of the liver patients received only diet and vitamins and had the above mentioned changes with continuation of the same therapy. These data may be interpreted as indicating the greater use of glucuronide conjugation mechanism in the acute phase of liver injury, possibly for the removal of endogenous metabolites that are not handled through their normal pathways. In that respect, it may parallel the increased glucuronide conjugation with benzoic or cinnamic acids as test materials when the primary methods of removal, glycine conjugation and oxidation, respectively, are impaired.

SUMMARY AND CONCLUSIONS

1. A method for the determination of serum cinnamate by measurement of a cadmium hydroxide filtrate in the ultraviolet at 270 mμ is presented.

2. Cinnamic acid administered orally in dosage of 0.6 gram per 30 lb. to normal subjects gave average serum cinnamic acid levels of less than 3.5 mg. per 100 ml. with conversion of 67 to 94 per cent hippuric acid in a six hour period. In patients with hepatocellular disease the serum cinnamic acid levels were elevated, ranging 4.2 to 13.8 mg. per 100 ml. with decreased conversion to hippuric acid and no significant changes in serum glucuronides, glucose, or inorganic phosphorus.

3. An important metabolic derangement in hepatic disorders, that of diminished oxidative capacity, may be conveniently measured with sodium cinnamate as the test substance. The rate of decline of serum cinnamate after intravenous injection with the dosage of 5 mg. per kg. can serve as an index of hepatic oxidative ability; the normal value is 4.0 to 6.0 per cent per minute. In hepatocellular damage the rate is decreased to as low as 0.8 per cent per minute and as seen from serial determinations is in direct relation to the clinical state of the patient. In 125 tests performed, minor side effects such as flushing and transient abdominal pain were occasionally present. The presence of jaundice interferes neither with the method of analysis nor the metabolic pathway. The indications are that the results of this test may have clinical usefulness in the evaluation of the extent of liver injury in conjunction with other laboratory aids.

4. Increased rates of removal of cinnamate from serum may occur in multiple myeloma as observed in two patients.

5. Patients with liver damage had a significant elevation of the concentration of serum glucuronic acid in the fasting state as compared with normal controls. With improvement in the clinical course of the liver disturbance there was a return of fasting glucuronic acid level to the normal.

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CEREBRAL CIRCULATION AND METABOLISM IN PULMONARY EMPHYSEMA AND FIBROSIS WITH OBSERVATIONS ON THE EFFECTS OF MILD EXERCISE[1]

By P. SCHEINBERG, I. BLACKBURN, M. SASLAW, M. RICH, AND G. BAUM

(From the Department of Physiology, University of Miami School of Medicine, the Department of Medicine, Veteran's Administration Hospital, Coral Gables, Fla., and the National Children's Cardiac Hospital, Miami, Fla.)

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Chronic pulmonary disease, particularly emphysema and fibrosis, may result in variable alterations in blood oxygen and carbon dioxide tensions, depending upon the severity of the disease. Changes in carbon dioxide and oxygen tensions have been shown to have a profound influence on cerebral circulation in acute experiments on normal subjects (1). Recently Patterson, Heyman, and Duke (2) demonstrated that cerebral blood flow was increased above normal in a group of subjects with severe chronic pulmonary emphysema, most of whom had cor pulmonale and were in congestive heart failure. Their studies re-emphasized the importance of the influence of blood carbon dioxide and oxygen tensions on cerebral blood flow, even though the alterations in the tensions of these gases were chronic. Their patients all had advanced pulmonary disease, however, and it was felt that it would be of interest to study the effects of less severe pulmonary disease, and less profound and more variable alterations in blood gas tensions, on the cerebral physiology. Most of these patients were studied before and during moderate exercise, since mental status changes are known to occur in some patients with chronic pulmonary disease during exertion.

METHODS

Cerebral blood flow studies were carried out by means of a modification (3) of the original nitrous oxide technique (4) on 9 of 22 subjects with chronic pulmonary disease. The original nitrous oxide technique with intermittent sampling was used in the other 13 patients. Twenty of the patients had chronic pulmonary emphysema of varying severity, confirmed by history, physical examination, radiographic and fluoroscopic studies, and, in most cases, by an estimation of maximal breathing capacity, vital capacity, and air velocity index (5). Two patients had pulmonary fibrosis without evidence of significant obstructive phenomena in the lungs, and this was confirmed by the finding of a normal air velocity index in these two subjects (S. A. and J. G., Table I). Electrocardiograms were made on all subjects but one (G. W.), and showed confirmatory evidences of cor pulmonale in 10 of the subjects. Eight of the subjects with cor pulmonale showed evidences of heart failure (though only one, C. A., was in severe failure) at the time of admission. The diagnosis of cor pulmonale in these patients was made by clinical findings, electrocardiographic changes, and chest fluoroscopy (evidences of enlargement of the right ventricular outflow tract). The patients were not studied until clinical evidences of congestive failure had disappeared on appropriate management, and none of the patients was studied during the acute stages of the illnesses which precipitated their admission, usually bronchiolar or pulmonary infections. In this way an effort was made to study each patient in a reasonably stable state, so that the possible effects of infection or heart failure would not interfere with an accurate interpretation of the results. The patients were classified for severity of pulmonary involvement according to the criteria set forth by Baldwin, Cournand, and Richards (6, 7).

The patients were fasting but no restrictions were placed on smoking prior to the procedure. In 12 of the subjects after the initial blood flow procedure was performed, exercise was carried out in the supine position by means of a type of exercycle attached to the foot of the table and supplied with variable resistance loads. This proved to be an unsatisfactory type of exercise, for in almost every instance the patient's legs would become tired before dyspnea appeared; many subjects who were unable to walk 50 yards without considerable dyspnea experienced little difficulty, even after performing the supine exercise for 20 to 35 minutes. The second blood flow was done after the patient had exercised for at least 10 minutes and the exercise was continued until the final blood samples were drawn. In one subject (T. K.) this exercise was sufficient to produce mild cyanosis, but none complained of severe respiratory distress, and none showed evidences of mental disturbances following exercise.

Arterial and venous oxygen contents were determined spectrophotometrically, according to the method of Hickam

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TABLE I

Clinical and pulmonary function data

Pt.	Age	Diagnosis and comments	Per cent normal maximal breathing capacity	Per cent normal vital capacity	Air velocity index	Classification
C. M.	59	Bronchial asthma, severe; pulmonary emphysema; no failure on admission.	30	42	0.72	I
S. A.	72	Tuberculosis, moderately advanced, active; pulmonary fibrosis; no failure on admission.	60	59	1.0	I
R. C.	56	Chronic bronchitis, pulmonary emphysema; cor pulmonale; no failure on admission.	28	41	0.66	IV
S. G.	58	Pulmonary emphysema; cor pulmonale; failure on admission.	27	35	0.77	IV
J. G.	55	Chronic bronchitis; pulmonary fibrosis; no failure on admission.	55	53	1.06	I
M. E.	58	Pulmonary granulomatosis; cor pulmonale; failure on admission.	—	—	—	IV
L. S.	40	Bronchial asthma, severe; pulmonary emphysema; no failure on admission.	38	52	0.73	I
N. S.	63	Pulmonary emphysema; cor pulmonale; failure on admission.	28	84	0.33	IV
H. J.	54	Intrinsic asthma; pulmonary emphysema; no failure on admission.	35	64	0.55	III
W. G.	53	Bronchial asthma; bronchiectasis; pulmonary emphysema; cor pulmonale; failure on admission.	38	49	0.78	IV
T. B.	50	Pulmonary emphysema; cor pulmonale; failure on admission.	25	36	0.69	IV
G. W.	41	Pulmonary emphysema; no failure on admission.	—	—	—	II
T. K.	56	Bronchial asthma; pulmonary emphysema; cor pulmonale; no failure on admission.	—	43	—	II
J. H.	25	Pulmonary emphysema and fibrosis; cor pulmonale; failure on admission.	36	—	—	IV
C. A.	57	Pulmonary emphysema; cor pulmonale; failure on admission.	24	56	0.44	IV
H. McD.	63	Bronchial asthma; pulmonary emphysema; no failure on admission.	—	43	—	I
G. C.	54	Bronchial asthma; pulmonary emphysema; no failure on admission.	42	75	0.56	II
W. E.	54	Pulmonary emphysema; no failure on admission.	60	79	0.76	II
R. S.	47	Bronchial asthma; pulmonary emphysema; no failure on admission.	55	99	0.56	II
N. V.	57	Pulmonary emphysema; cor pulmonale; failure on admission.	21	36	0.58	IV
C. K.	55	Bullous emphysema; no failure on admission.	—	—	—	III
L. S.	74	Bronchial asthma; pulmonary emphysema; no failure on admission.	—	—	—	II

and Frayser (8). Blood samples for oxygen determination were drawn before and after each blood flow measurement, and the average was used as the final value for arterial-cerebral venous oxygen difference and arterial oxygen saturation. Carbon dioxide determinations were carried out on the van Slyke-Neill manometric apparatus, as described by Peters and van Slyke (9). Arterial blood pressure was recorded at heart level by the auscultatory method at intervals of approximately two minutes, and mean pressure calculated from the formula: MP = diastolic pressure + ⅓ pulse pressure. The calculated mean pressures checked closely with mean pressures measured directly by means of a mercury U-tube manometer.

pH measurements were made with the Model R Cambridge pH meter. Blood was drawn into heparin-coated syringes and immediately transferred to a constant temperature room at 33–34° C. The syringes were allowed to stand for three to six minutes to allow the temperature to fall from body temperature to the temperature of the room before readings were made. Buffers at pH 4.00 (acid phthalate) and 6.99 (phosphate) were used to check the instrument for standardization. The Rosenthal factor was used to correct the readings to 38° C. (10). Arterial and venous oxygen tensions were estimated from the pH and the per cent oxyhemoglobin saturation, using the dissociation curves of Dill. The values for oxygen tension when oxyhemoglobin saturation was above 90 per cent were obtained from a chart of interpolated values, using Dill's figures, since it was impossible to read the curves accurately above this point. Carbon dioxide ten-

TABLE II

Cerebral vascular and metabolic functions in chronic pulmonary disease with observations on exercise

Patient	Arterial O_2 tension		CBF		O_2 content				A-VO_2		Arterial CO_2 content		Arterial pH		Arterial CO_2 tension		Per cent O_2 saturation				Cerebral venous O_2 tension		$CMRO_2$		Mean arterial pressure		CVR	
					Arterial		Venous										Arterial		Venous									
	mm. Hg		ml./min./100 gm. brain		vol. %		vol. %		vol. %		vol. %				mm. Hg						mm. Hg		ml. O_2/min./100 gm. brain		mm. Hg		mm. Hg/ml. blood/min./100 gm. brain	
	a	p	a	p	a	p	a	p	a	p	a	p	a	p	a	p	a	p	a	p	a	p	a	p	a	p	a	p
C. M.	76	71	44	49	16.7	16.9	7.3	10.2	9.4	6.7	45.1	45.9	7.39	7.35	39	43	94	92	41	55	24	29	4.1	3.3	89	99	2.02	2.02
S. A.	78	86	33	53	17.5	17.3	9.4	10.6	8.2	6.7	41.0	45.7	7.40	7.36	37	42	95	95	50	59	28	33	2.7	3.6	104	114	3.15	2.15
R. C.	85	72	59	53	16.9	16.5	12.3	11.7	4.6	4.8	56.3	55.5	7.37	7.37	50	49	95	93	69	66	36	34	2.7	2.6	116	126	1.97	2.38
S. G.	81	88	42	43	17.0	17.4	9.2	8.0	7.8	8.0	68.0	62.6	7.40	7.40	54	51	95	96	51	52	29	29	3.3	3.4	92	103	2.19	2.40
J. G.	69	69	64	65	17.6	17.4	10.7	10.5	6.9	6.9	48.4	49.3	7.39	7.38	41	43	92	92	56	56	33	32	4.4	4.5	90	94	1.41	1.45
M. E.	—	—	48	—	21.6	—	14.5	—	7.1	—	—	—	—	—	—	—	96	—	67	—	35	—	3.4	—	74	—	1.50	—
L. S.*	77	85	51	43	18.7	19.9	10.8	11.9	8.0	8.0	49.2	47.8	7.35	7.34	45	45	94	95	54	57	32	33	4.1	3.4	80	94	1.57	2.18
N. S.*	69	60	86	77	17.3	16.7	14.2	12.8	3.1	3.8	68.8	68.2	7.37	7.37	60	59	92	88	76	67	43	38	2.6	2.9	71	68	0.83	0.88
H. J.*	73	—	62	—	15.5	—	11.0	—	4.6	—	53.5	—	7.34	—	50	—	92	—	65	—	37	—	2.8	—	98	—	1.58	—
W. G.*	66	89	39	44	18.6	19.0	10.7	10.8	7.8	8.1	54.1	51.3	7.40	7.36	45	51	92	95	53	55	31	33	3.0	3.5	87	124	2.23	2.82
T. B.*	51	51	55	48	14.3	13.5	7.9	8.0	6.4	5.5	—	—	7.35	7.30	—	—	82	80	46	48	28	30	3.5	2.6	90	88	1.64	1.83
G. W.*	85	—	63	—	17.5	—	11.7	—	5.8	—	50.3	—	7.30	—	51	—	94	—	63	—	37	—	3.7	—	77	—	1.20	—
T. K.*	73	54	60	107	18.7	16.3	12.5	13.6	6.3	2.7	53.5	55.8	7.43	7.26	41	62	94	81	67	66	35	42	3.8	2.9	91	98	1.52	0.92
J. H.*	49	—	52	—	20.5	—	12.2	—	8.3	—	—	—	7.36	—	—	—	80	—	48	—	29	—	4.3	—	84	—	1.61	—
C. A.*	52	50	58	66	17.2	16.1	13.1	12.1	4.1	4.0	—	—	7.36	7.32	—	—	83	80	63	60	36	35	2.4	2.7	94	113	1.62	1.71
H. McD.*	63	—	39	—	18.0	—	9.8	—	8.2	—	49.0	—	7.42	—	42	—	92	—	50	—	29	—	3.2	—	119	—	3.05	—
G. C.*	72	—	73	—	16.1	—	9.9	—	6.3	—	51.8	—	7.35	—	47	—	92	—	56	—	33	—	4.6	—	85	—	1.16	—
W. E.*	66	—	47	—	15.4	—	9.5	—	5.9	—	47.6	—	7.37	—	43	—	91	—	55	—	31	—	2.8	—	92	—	1.96	—
R. S.*	73	70	42	52	16.4	16.8	11.3	11.4	5.1	5.3	50.6	49.2	7.36	7.34	46	47	92	91	64	62	37	36	2.2	2.8	84	93	2.00	1.79
N. V.	77	—	49	—	17.8	—	9.0	—	8.8	—	49.9	—	7.37	—	44	—	94	—	47	—	27	—	4.3	—	84	—	1.71	—
C. K.	62	—	54	—	18.4	—	7.3	—	7.3	—	49.1	—	7.39	—	43	—	90	—	54	—	30	—	4.0	—	74	—	1.37	—
L. S.	60	—	55	—	16.9	—	11.2	—	5.7	—	51.4	—	7.36	—	46	—	88	—	59	—	34	—	3.1	—	93	—	1.69	—
Mean	69.4		53.4		17.5		10.7		6.62		52.1		7.37		45.8		91.3				32.5		3.41		89.5		1.77	
Stand. devia.	10.4		12.1		1.6		2.0		1.7		6.9		0.03		5.6		4.4				4.4		0.7		12.1		0.5	
Stand. error	2.3		2.6		0.3		0.4		0.4		1.6		0.006		1.3		0.9				0.9		0.2		2.6		0.1	
Normals			55.3						6.58								96.2				32.3		3.64		96.5		1.82	
Stand. error			1.97						0.18								0.21				.71		0.15		2.16		0.08	
p value			>0.2						0.2								<0.01				>0.5		>0.5		>0.2		>0.5	

* Indicates subjects in whom intermittent sampling technique was used.
a = before exercise.
p = during exercise.

sions were estimated from the nomograms of Peters and van Slyke (9).

RESULTS

Comparison with normal subjects. A clinical summation of the patients is given in Table I, and the results of the studies are given in detail in Table II. The patients ranged in age from 25 to 74 years, with an average of 55 years. They were compared with a group of 32 normal subjects who ranged in age from 38 to 79 years, averaging 56 years (11). It was felt necessary to compare the subjects with others in a similar age group, as definite changes in cerebral circulation and metabolism accompany advancing age (11). There was no significant alteration in cerebral blood flow, arterial-cerebral venous O_2 difference, cerebral O_2 consumption, or cerebral vascular resistance (Table II).

The blood gas tensions indicate that the chemical effects of pulmonary disease in most of these patients were not extensive. Only five of the patients had values for pCO_2 of 50 mm. Hg or above; however, pCO_2 determinations were not obtained in the three patients with the lowest arterial O_2 saturation and tensions because of technical error. Were these figures available, it is possible that a much higher mean for pCO_2 would have been obtained, but even so the mean value for cerebral blood flow is within normal limits. Arterial pH did not differ significantly from the normal, and mean per cent arterial oxygen saturation was 91.3, both indications that this group represents relatively mild, or at least well-compensated pulmonary disease.

If the subjects are divided into two groups according to clinical severity of their disease, by contrasting those subjects in group I and II with those in groups III and IV, there are no significant differences observed in cerebral blood flow, cerebral oxygen consumption, and arterial pH between the two groups. Since the clinical classification is itself partially dependent upon arterial pO_2 and pCO_2 values, the value for pO_2 is significantly lower and that for pCO_2 higher in the second

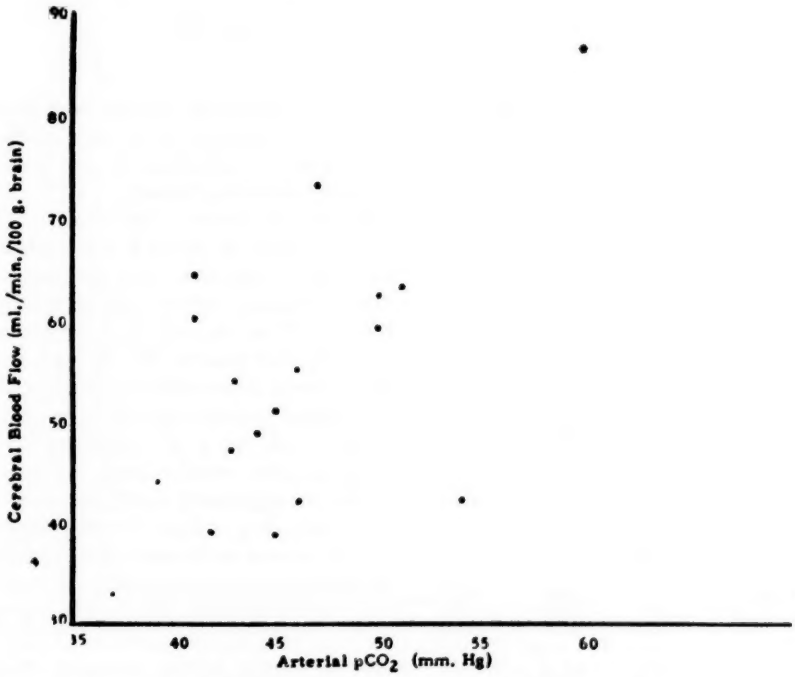

FIG. 1. CORRELATION BETWEEN ARTERIAL pCO_2 AND CBF
$r = 0.63$ $p < 0.01$

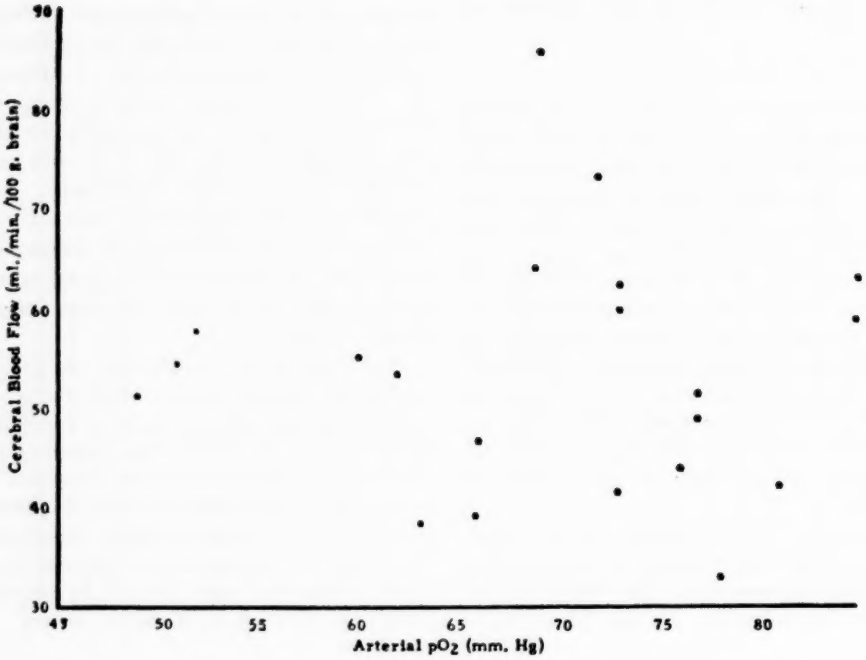

FIG. 2. CORRELATION BETWEEN ARTERIAL pO_2 AND CBF
$r = 0.03$

group than in the first. When considering all the patients together, however, there is an adequate correlation between arterial pCO_2 and cerebral blood flow and no correlation between arterial pO_2 and cerebral blood flow (Figures 1 and 2). There is also no correlation between cerebral blood flow and clinical severity of the ventilatory defect as expressed by reduction in percentage normal maximal breathing capacity.

TABLE III

Effects of mild exercise on cerebral metabolic functions (mean values in 12 subjects)

	Before exercise	During exercise	p value
Art. pO_2	70.8	70.4	>0.5
CBF	52.8	58.3	>0.2
Art. O_2 content	17.2	17.0	>0.5
Venous O_2 content	10.8	11.0	>0.5
A-V O_2	6.48	5.88	>0.1
Art. CO_2 content	53.5	53.1	>0.5
Venous CO_2 content	54.7	54.2	>0.5
Art. pH	7.38	7.34	<0.02*
Art. pCO_2	45.8	49.2	>0.2
Venous pCO_2	50.1	51.1	>0.5
Per cent art. O_2 sat.	91.7	89.8	>0.2
Per cent venous O_2 sat.	57.5	58.6	>0.5
Venous pO_2	32.7	33.7	>0.05
$CMRO_2$	3.23	3.18	>0.5
MAP	90.7	101.2	<0.01*
CVR	1.85	1.88	>0.5

* Denotes statistically significant change.

Effects of exercise. The effects of exercise on the 12 subjects in whom it was studied are summarized in Table III. The only statistically significant changes were the drop in arterial pH from 7.38 to 7.34 and the increase in mean arterial pressure. It is of interest that the only two subjects who showed a considerable rise in cerebral blood flow during exercise also had a sizable increase in pCO_2 (S. A. and T. K., Table II), although two other subjects showed some increase in pCO_2 without accompanying significant elevation of cerebral blood flow. There is no evidence of even a trend toward an increase in cerebral blood flow or decrease in arterial-cerebral venous O_2 difference during exercise, for the changes were quite variable, and only one patient (T. K.) was actually responsible for the apparent change in the mean values of these functions. Cerebral oxygen consumption and cerebral vascular resistance were

not altered by exercise, although there was a fairly consistent increase in mean arterial pressure. There was no consistent change in per cent arterial O_2 saturation or arterial pO_2, the only striking reduction in either occurring in T. K. This is further evidence of the relative mildness of this type of exercise or the relative lack of severity of pulmonary disease in these patients, or both.

Pulmonary fibrosis. The two patients with pulmonary fibrosis showed no differences in cerebral metabolic functions from the patients with emphysema.

Intermittent vs. continuous sampling techniques. There was no significant difference in any of the cerebral metabolic functions in the nine subjects studied by the continuous sampling technique as compared to the 13 studied by the original intermittent sampling technique, as seen in Table IV, and for that reason both groups were combined for final statistical analysis in this study. The studies were done by both techniques as a part of planned observations to determine the possible reason for the differences in mean values in normal subjects as reported by Kety and Schmidt (4), Scheinberg and Stead (3) and Patterson, Heyman, and Nichols (12). In this group of subjects, at least, there is no significant difference between results obtained by the two techniques.

TABLE IV

Comparison of intermittent and continuous sampling techniques (mean values)

	Intermittent (13 subjects)	Continuous (9 subjects)
CBF	57	51
$CMRO_2$	3.3	3.5
CVR	1.69	1.89

All p values >0.3.

Electroencephalograms. Electroencephalograms performed on eight of the subjects were all within normal limits.

DISCUSSION

The data reported here cannot be compared with the observations on cerebral circulatory functions in pulmonary emphysema reported by Pat-

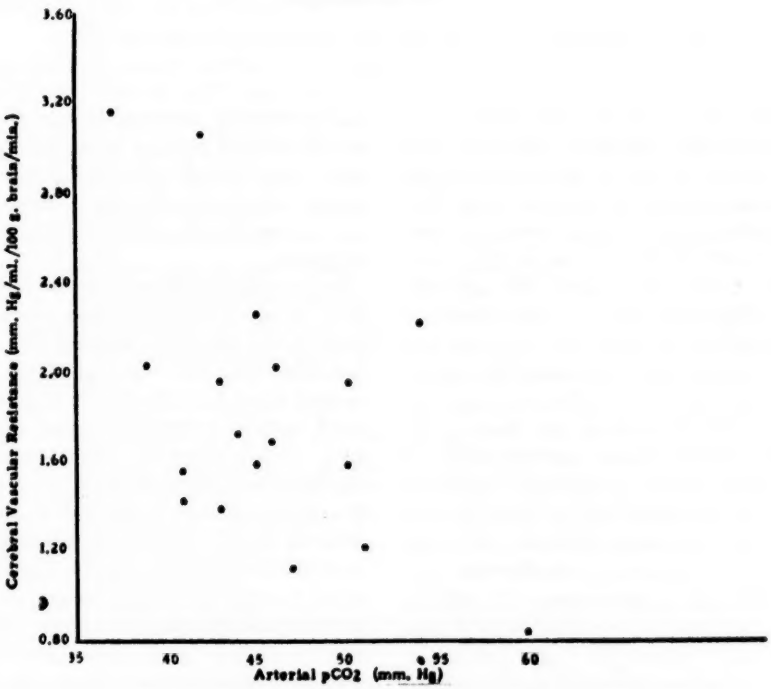

FIG. 3. CORRELATION BETWEEN CVR AND ARTERIAL pCO_2

$r = 0.51$ $\quad 0.05 > p > 0.01$

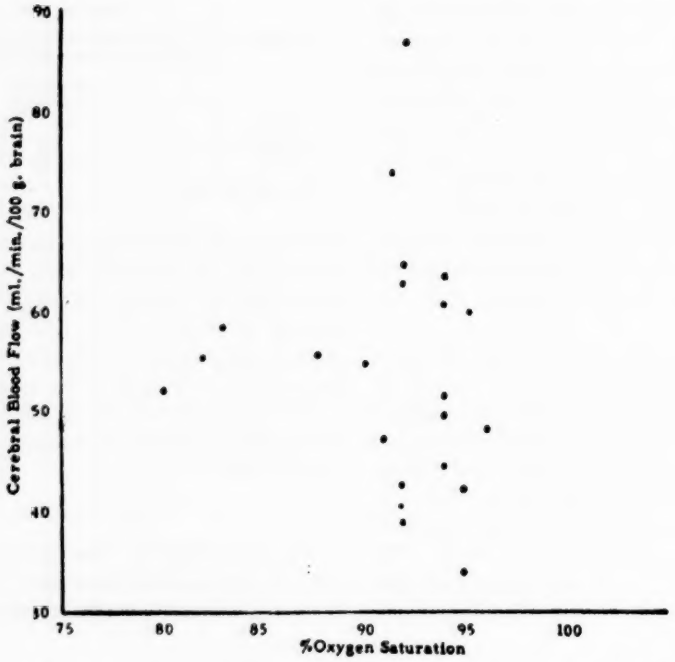

FIG. 4. CORRELATION BETWEEN PER CENT OXYGEN SATURATION AND CBF
$r = 0.03$

terson, Heyman, and Duke (2), since most of our subjects suffered less pulmonary disability than did theirs. In both groups of subjects there was adequate correlation between cerebral blood flow and pCO_2, and cerebral vascular resistance and pCO_2. In our subjects the p values for these correlations were < 0.01 and between 0.01 and 0.05, respectively (Figures 1 and 3). We found no correlation, however, between pO_2 and cerebral blood flow or between per cent arterial O_2 saturation and cerebral blood flow (Figures 2 and 4), and we are unable to explain this discrepancy. Our patients were not studied until the effects of the acute illnesses which precipitated admission to the hospital had subsided and the patients were reasonably stable. Patterson, Heyman, and Duke make no statement concerning whether this precaution was observed in their group. In addition five of the nine patients they reported had evidence of congestive heart failure due to cor pulmonale. Since this type of failure reportedly is accompanied frequently by an increased cardiac output (13), there is a possibility that the failure itself rather than pulmonary emphysema may be responsible for the elevated cerebral blood flow in these subjects. Two of their subjects had low and normal cardiac indices (T-1824 dye), however, making such an explanation dubious without further investigation.

The correlation between cerebral blood flow and pCO_2 in our subjects confirms previous observations on the effects of inspired CO_2 on cerebral circulation (1), and the normal mean value for cerebral blood flow obtained in this group is compatible with an essentially normal mean value for pCO_2. The absence of correlation between cerebral blood flow and arterial pO_2 is surprising, as is the apparent absence of effect of a decreased mean value for arterial pO_2 in the whole group. Previous observations (1) have indicated that a decrease in pO_2 is a fair stimulus for producing cerebral vasodilatation in normal subjects; perhaps the absence of this phenomenon in the group of subjects reported here indicates the development of a tolerance and diminished response to the cerebral vasodilating effect of a low oxygen environ-

ment if the oxygen deprivation is chronic rather than acute.

Little was learned about the effects of exercise in this study, except that patients with only moderately severe functional pulmonary disability have a remarkable tolerance to this type of exercise. One might anticipate an increase in cerebral blood flow to accompany a rise in arterial pCO_2 in those patients whose limitation of ventilatory capacity prevents compensatory hyperventilation, and such seemed to be the case in the two subjects who showed the most striking chemical responses to exercise. It is of interest that the reduction in arterial pH during exercise was not associated with a change in cerebral blood flow. This is further evidence that alteration in arterial pH per se is not the important factor in the regulation of cerebral blood flow (14). Since none of our subjects developed signs of mental confusion during this relatively mild exertion, one of the purposes of this study, which was to investigate the physiological basis for the confusion which occurs during physical exertion in many such patients, was not achieved.

These studies verify the previously established relationship between arterial pCO_2 and cerebral blood flow and demonstrate a relative lack of importance of individual changes in arterial pH, arterial pO_2, and arterial CO_2 content in the regulation of cerebral circulation. They also demonstrate that cerebral vascular and metabolic functions in chronic pulmonary diseases are unrelated to the severity of the clinical and spirometric disturbance, but are rather related to alteration in gaseous exchange in the lungs, and specifically to carbon dioxide retention.

SUMMARY

1. Cerebral blood flow, oxygen consumption, and vascular resistance were measured in 22 patients with chronic pulmonary disease of moderate functional severity, and compared with a group of normal controls of the same average age. The effects of mild exercise were studied in 12 of the patients.

2. There was no significant difference in cerebral blood flow as measured by the intermittent sampling technique in 13 of these subjects as compared to the continuous sampling technique in the remaining nine subjects.

3. Mean values for cerebral blood flow, cerebral A-V O_2 difference, cerebral oxygen consumption, and cerebral vascular resistance did not differ significantly from the normal control subjects.

4. The correlation between cerebral blood flow and arterial pCO_2 was good, whereas no correlation was found between cerebral blood flow and arterial pO_2 or per cent arterial O_2 saturation.

5. The effects of exercise on cerebral metabolic functions in these patients were variable and without a significant trend. A reduction in arterial pH during exercise did not influence cerebral blood flow.

6. These studies re-emphasize the important relationship between arterial pCO_2 and cerebral blood flow and demonstrate the relative unimportance of the clinical and spirometric severity classification on cerebral blood flow in chronic pulmonary disease.

ACKNOWLEDGMENT

The authors gratefully acknowledge the technical assistance of Mrs. Janet Fetner.

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CHANGES IN ACID–BASE BALANCE OF UREMIC PATIENTS DURING HEMODIALYSIS [1]

By JOHN M. WELLER,[2] ROY C. SWAN,[3] AND JOHN P. MERRILL [4]

(From the Department of Medicine, Peter Bent Brigham Hospital and Harvard Medical School, Boston, Mass.)

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Patients who are acutely or chronically ill with the uremic syndrome are clinically improved following the procedure of external hemodialysis. The exact changes responsible for this improvement are not known. It has been shown that dialysis removes retained nitrogenous metabolites and returns disordered serum electrolyte concentrations toward normal (1). The purpose of this study is to describe more precisely the changes in the acid-base balance present in uremic patients and their modification during hemodialysis.

MATERIALS AND METHODS

Ten patients having renal insufficiency were studied. One of these (D. B.) had acute renal failure due to paroxysmal myoglobinemia. On the day prior to hemodialysis, which was carried out on the ninth day of anuria, he was given 10 gms. of sodium bicarbonate intravenously. The other nine patients had chronic renal insufficiency. One (H. M.) had chronic glomerulonephritis; one (M. K.) had nephrosclerosis, and the others had chronic pyelonephritis. Four patients had received sodium bicarbonate orally prior to dialysis. Two (M. L. and F. M.) had 3 gms. daily for one week; one (E. C.) had 2 gms. daily for five days, and one (B. L.) had 12.5 gms. daily for three days.

Hemodialysis was applied to all patients by means of a modified Kolff-type artificial kidney (2). The dialysate bath in all cases contained per liter of bath 140 mEq. sodium, 4 mEq. potassium, 2.5 mEq. calcium, and 1.0 mEq. magnesium. In eight cases the bath chloride was 120 mEq. per L. and the bicarbonate 27 mEq. per L., while in two cases (D. B. and D. G.) the chloride was 112 mEq. per L. and the bicarbonate 35 mEq. per L. The bath was maintained at approximately pH 7.4 by equilibration with a 5 or 10 per cent carbon dioxide gas phase. The duration of dialysis was six hours in each case except one (E. G.) in which it was four hours. The bath was completely changed every two hours. Arterial samples of plasma, serum, or whole blood collected anaerobically were analyzed for serum pH by the method of Hastings and Sendroy (3); total CO_2 content, Van Slyke and Neill (4); blood urea nitrogen, Archibald (5); serum sodium and potassium with a Barclay flame photometer using lithium as an internal standard; serum chloride, Schales and Schales (6); serum phosphorus, Fiske and Subbarow (7); and serum calcium, Clark and Collip (8).

RESULTS

The determined values of the percentage of packed red cells, Hct., the serum pH, pH_s and the serum total CO_2 content, $(CO_2)_s$, are shown in Table I. Also given in Table I are the determined values of the blood urea nitrogen and the serum electrolytes including the serum total inorganic phosphorus in those cases in which it was analyzed. These values are shown for both the onset of dialysis (0 hour) and the end of dialysis (6 hours).

Calculated values of the components which delineate the acid-base balance are shown in Table II. The buffer base of the whole blood, $(B_B^-)_b$, and the arterial (or alveolar) carbon dioxide pressure, P_{CO_2}, have been derived from the nomogram of Singer and Hastings (9). Their definitions of terms are used in this presentation. The buffer base is the calculated concentration of base equivalent to the sum of the concentrations of the buffer anions. The principal buffer anions are protein (both the hemoglobin of the red cells and the plasma proteins) and bicarbonate. In addition to these anions, a portion of the phosphate is likewise effective as a buffer. The buffer base is also equal to the difference between the sum of the cations (total base) and the sum of the non-buffer anions (chloride and other "fixed acids"). The

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[2] Instructor in Medicine, Harvard Medical School; Junior Associate in Medicine, Peter Bent Brigham Hospital.

[3] Life Insurance Medical Research Fellow.

[4] Established Investigator, American Heart Association, Associate in Medicine, Harvard Medical School and Peter Bent Brigham Hospital.

TABLE I

Determined values at the beginning and end of hemodialysis

Patient	Hct. %		pH_a units		$(CO_2)_a$ mM/L.		$(Na^+)_a$ mEq./L.		$(K^+)_a$ mEq./L.		$(Cl^-)_a$ mEq./L.		Inorg. P_a mM/L.		BUN mg./100 ml.	
	0 hr.	6 hrs.	0 hr.	6 hrs.	0 hr.	6 hrs.	0 hr.	6 hrs.	0 hr.	6 hrs.	0 hr.	6 hrs.	0 hr.	6 hrs.	0 hr.	6 hrs.
B. L.	24.6	22	7.32	7.48	15.8	20.8	127	144	3.9	4.4	92	116	4.5	1.6	215	76
E. G.*	37	33	7.32	7.38	12.8	19.4	124	130	6.7	5.3	92	99	—	—	145	63
M. L.	28	28	7.22	7.34	23.4	25.2	152	164	5.3	4.9	106	132	2.7	—	205	80
F. M.	30.2	27.3	7.22	7.37	17.6	24.0	135	135	3.9	4.4	96	120	1.5	0.8	157	55
E. C.	29	23	7.13	7.42	12.9	21.4	129	140	4.8	4.0	101	107	—	—	140	27
M. K.	19	19	7.32	7.53	12.8	23.3	119	135	7.3	5.4	77	94	5.2	1.7	291	87
D. B.	40	37	7.18	7.32	16.5	18.5	129	145	5.6	3.9	76	97	4.0	1.6	207	108
D. G.	27	25	7.37	7.70	9.4	22.9	123	140	5.4	3.9	87	107	6.9	1.8	313	76
C. M.	16	17	6.90	7.42	9.9	20.3	133	143	5.0	4.1	100	115	—	—	355	116
H. M.	18	27	6.97	7.26	4.5	8.9	133	141	7.2	5.5	85	—	—	—	308	134

* 4 hour values instead of 6 hour.

total base concentration and the non-buffer anion concentration are not directly altered by changes in respiration but are directly altered by changes in renal or gastro-intestinal excretion. These non-respiratory changes are termed "metabolic" by Singer and Hastings. A primary metabolic disturbance producing either a decrease in total base concentration or an increase in non-buffer anion concentration results in a decrease in the buffer base concentration. This is termed a primary metabolic acidosis.

The carbon dioxide pressure of the arterial blood, P_{CO_2}, is in equilibrium with the alveolar carbon dioxide pressure. This may be rapidly altered by changes in pulmonary ventilation. A primary change in pulmonary ventilation which decreases the P_{CO_2} is termed a primary respiratory alkalosis, while an increase in P_{CO_2} is a respiratory acidosis.

A primary change in either the buffer base or the carbon dioxide pressure is usually accompanied by a secondary change in the other variable. These secondary or "compensatory" changes are in the direction which minimizes the deviation of the serum pH. The selection of the primary change when mixed disturbances are present is based upon evaluation of the clinical situation.

Also given in Table II are the calculated concentrations of both the buffer and non-buffer serum anions. The serum bicarbonate ion concentration, $(HCO_3^-)_a$, and the serum protein ion concentration, $(Prot^-)_a$, have been derived from the nomogram of Singer and Hastings; the former directly, while the latter is the difference between the serum buffer base concentration, which is obtained directly from the nomogram, and the serum bicarbonate ion concentration. That fraction of the serum phosphate which is available as a buffer anion, $(HPO_4^-)_a$, has been calculated from the determined total phosphate using the correspond-

TABLE II

Derived values at the beginning and end of hemodialysis

Patient	$(B_B^+)_b$ mEq./L.		PCO_2 mm. Hg		$(HCO_3^-)_a$ mEq./L.		$(Prot^-)_a$ mEq./L.		$(HPO_4^-)_a$ mEq./L.		$(B^+)_a$ mEq./L.		$(A^-)_a$ mEq./L.	
	0 hr.	6 hrs.	0 hr.	6 hrs.	0 hr.	6 hrs.	0 hr.	6 hrs.	0 hr.	6 hrs.	0 hr.	6 hrs.	0 hr.	6 hrs.
B. L.	35.2	42.0	29.4	27.1	14.9	20.0	16.8	18.2	7.3	2.8	131	148	92†	107†
E. G.*	34.3	40.8	23.8	31.5	12.2	18.3	16.8	17.5	—	—	131	135	102	99
M. L.	40.0	44.0	53.8	45.1	21.7	23.8	16.1	17.0	4.2	—	157	169	119	128
F. M.	35.5	43.5	40.8	39.8	16.3	22.8	16.1	17.2	2.3	1.3	139	139	104†	98†
E. C.	29.8	41.8	36.0	32.3	11.8	20.4	15.7	17.9	—	—	134	144	106	106
M. K.	31.8	44.3	23.8	27.3	12.1	22.5	16.8	18.8	8.4	2.8	126	140	89†	96†
D. B.	34.8	39.2	41.5	34.6	15.2	17.4	16.0	16.9	6.0	2.6	135	149	98†	112†
D. G.	30.3	47.9	15.5	18.4	8.9	22.3	16.9	20.3	11.4	3.3	128	144	91†	98†
C. M.	22.1	39.7	45.1	30.8	8.5	19.3	13.5	18.0	—	—	138	147	116	110
H. M.	18.7	28.0	17.8	18.6	4.0	8.3	14.2	16.0	—	—	140	147	122	123

* 4 hour values instead of 6 hours.

† $(HPO_4^-)_a$ included in calculation.

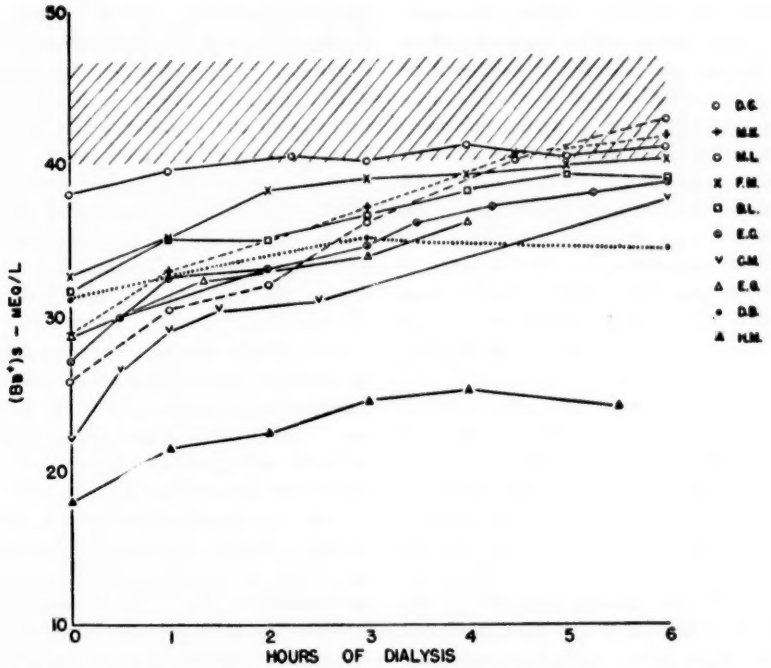

FIG. 1. THE CHANGES WITH TIME OF THE SERUM BUFFER BASE OF 10 PATIENTS DURING HEMODIALYSIS

The diagonally hatched area represents the normal range of the serum buffer base.

ing determined serum pH. This fraction of the serum phosphate anion, the serum protein anion, and the serum bicarbonate anion together constitute the serum buffer anions.

The total serum base concentration is equal to the sum of the concentrations of the serum cations. Sodium is the principal serum cation. The calculated total serum base, $(B^+)_s$, given in Table II, is the sum of only the sodium and potassium. Calcium and magnesium are not included. The serum calcium was determined at both 0 and 6 hours in four cases with the following results: M. L., 3.2 to 5.0; M. K., 3.9 to 4.1; D. B., 2.5 to 3.0, and D. G., 2.7 to 3.6 mEq. per L. The concentration of total serum non-buffer anions, or "fixed acids," $(A^-)_s$, is calculated by subtracting from the total serum base the sum of the serum buffer anions. Chloride constitutes the major portion of the non-buffer serum anions. The phosphate fraction of the serum buffer anions has been omitted from this calculation in those cases in which it was not determined at both 0 and 6 hours.

Acid-base balance prior to dialysis

All patients except one (D. G.) were "acidotic" (pH_s lower than 7.35) prior to the commencement of hemodialysis. In two patients there was a marked lowering of the serum pH to below 7.0. All cases had a concentration of whole blood buffer base prior to dialysis which was below the lower limit of normal (45 mEq. per L.). Their acidosis was, therefore, of the metabolic type, and because all patients had renal insufficiency, this metabolic acidosis is considered to be the primary disturbance in the acid-base balance. The decreased whole blood buffer base concentration was due to a diminution in both the protein and the bicarbonate anion fractions. A decreased hemoglobin concentration present in all but two patients (E. G. and D. B.), as evidenced by the lowered hematocrits, accounted for the major portion of the decrease in the protein fraction. The absence of anemia in these two patients was correlated with the shorter duration of their uremia. The serum protein frac-

tion of the buffer base was only slightly decreased. The lowered whole blood buffer base concentration prior to dialysis was also due to a decrease in the bicarbonate ion concentration as evidenced by the diminished concentration of serum bicarbonate. In all cases the serum bicarbonate was below the lower limit of normal (23 mEq. per L.). In contrast to the decrease in the concentration of the serum bicarbonate and protein anions there was an increase in the concentration of the buffer fraction of the serum phosphate anions due to renal retention of phosphate. This increase was not as great in magnitude as was the decrease in the principal buffer anions.

The decreased serum buffer base concentration may be the resultant of either a decrease in total serum base or an increase in the serum non-buffer anions. Eight patients had a concentration of total base below 140 mEq. per L. prior to dialysis. This decreased serum base was due to a diminished concentration of serum sodium. Only three patients prior to dialysis showed a significant increase (over 115 mEq. per L.) in the concentration of serum "fixed acids" (chloride and other non-buffer anions). Of the seven patients not having a "fixed acid" excess only one (E. C.) had a normal serum chloride concentration. The others had some degree of hypochloremia. The decrease in the serum chloride concentration of these patients offset the increase in the concentration of other "fixed acid" anions so that the total concentration of non-buffer anions was not increased. Of the three patients having a "fixed acid" excess, two (M. L. and C. M.) had normal serum chloride concentrations while the third (H. M.) had hypochloremia.

The primary metabolic acidosis present in these ten patients having renal insufficiency was, therefore, due to a decrease in buffer base concentration. This was associated with a total serum base deficit in eight patients while a "fixed acid" excess was present in only three.

Prior to dialysis, the arterial carbon dioxide pressure (P_{CO_2}) was normal (between 35 and 48 mm. Hg) in four cases, slightly elevated in one and reduced in five. The lowered CO_2 pressures in these five cases are considered to be secondary respiratory deviations (secondary respiratory

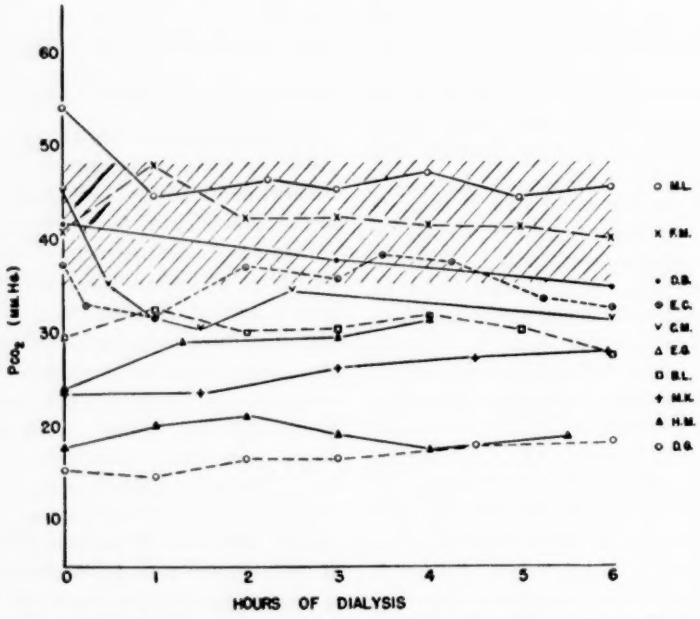

FIG. 2. THE CHANGES WITH TIME OF THE ARTERIAL CARBON DIOXIDE PRESSURE OF 10 PATIENTS DURING HEMODIALYSIS

The diagonally hatched area represents the normal range of the arterial carbon dioxide pressure.

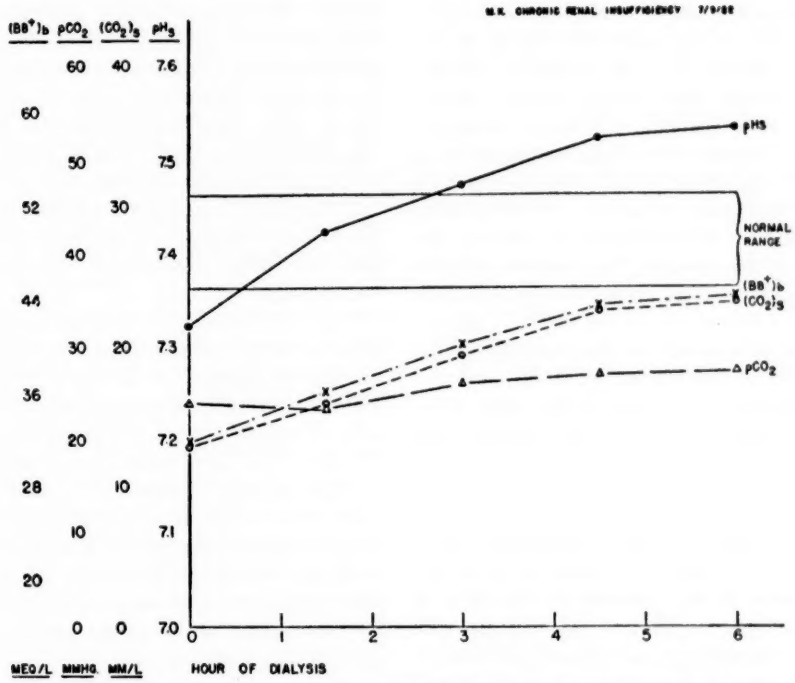

FIG. 3. CHANGES IN THE WHOLE BLOOD BUFFER BASE $(B_B^-)_b$; THE ARTERIAL CARBON DIOXIDE PRESSURE, (P_{CO_2}); THE SERUM TOTAL CO_2 CONTENT, $(CO_2)_s$, AND THE SERUM pH, pH_s, IN ONE PATIENT (M. K.) DURING HEMODIALYSIS

alkalosis). These five patients, therefore, had a mixed disturbance of the acid-base balance, primary metabolic acidosis with secondary respiratory alkalosis.

Changes in acid-base balance during dialysis

It can be seen in Table II that in all instances dialysis resulted in an increase in the whole blood buffer base concentration. This change was not due to an increase in the concentration of the hemoglobin anion fraction of the whole blood buffer base as only one patient (H. M.) showed a significant increase in hematocrit. A small increase in the base equivalents of the protein anions present did occur in association with the increase in pH. The major increase in the whole blood buffer base was accounted for by the increase in the bicarbonate ion fraction. In Figure 1 are shown the changes during the course of dialysis of the sum of the concentrations of the principal buffer anions of the serum (bicarbonate and protein). The serum phosphate fraction has not been included. As the serum protein anion fraction showed only a small change during dialysis, this increase in the serum buffer base was primarily due to an increase in the serum bicarbonate fraction. The rate of change of the serum buffer base is greater during the initial phase of dialysis.

The increase in the serum buffer base concentration was associated with an increase in the serum total base concentration in all but one case. The increase in the serum total base was due primarily to an increase in the serum sodium. In addition there was an increase in the concentration of the chloride fraction of the "fixed acids" in all instances, but at the same time, this increase was in part offset by a decrease in the concentration of other non-buffer anions. Dialysis, therefore, resulted in alleviation of the metabolic acidosis by increasing the whole blood buffer base, primarily by increasing the bicarbonate anion concentration. Dialysis also moderated both the serum total base deficit and the hypochloremia and decreased the serum concentration of other "fixed acids."

This prompt correction of the metabolic acidosis occurs without any consistent change in the respiratory component of the acid-base balance. The P_{CO_2} showed little change during dialysis (Figure 2). The patients that had a secondary respiratory alkalosis (low P_{CO_2}) at the onset of dialysis had persistence of this low arterial P_{CO_2} throughout the course of dialysis. As the primary metabolic acidosis was alleviated by dialysis, this persistence of the lowered P_{CO_2} resulted in three cases (B. L., M. K., and D. G.) in actual alkalosis (pH_a above 7.46) at the end of dialysis. Figure 3 shows the development of this alkalosis during dialysis in one of these patients (M. K.) and also shows the changes with time of the other determined and calculated values which describe the acid-base disturbance.

DISCUSSION

These ten patients with renal insufficiency were all seriously ill and were considered to be in the terminal phase of their disease at the time of dialysis. Indeed, all nine of the patients with chronic renal insufficiency subsequently succumbed. The one patient (D. B.) with acute renal insufficiency recovered. However, all of the patients except one (H. M.) showed a good clinical response to dialysis and survived for many weeks. The one patient who did not respond was moribund at the time of dialysis and died shortly after the termination of the procedure.

All of the patients dialyzed had a metabolic acidosis (decreased whole blood buffer base concentration). This was due to a decrease in both the protein (primarily hemoglobin) and bicarbonate fractions of the buffer base. Dialysis caused a slight increase in the protein fraction and a much larger increase in the bicarbonate fraction. Conservative treatment utilizing both whole blood or red cell transfusions and sodium bicarbonate or lactate will result in an increase in both fractions of the buffer base (10). Only one (H. M.)of the patients in this study received blood during dialysis. However, the use of the artificial kidney does not preclude the administration of blood either during the procedure or independently. Restoration of buffer base, therefore, occurs during both dialysis and conservative therapy.

All patients showed a decrease in total serum base concentration which was moderated by dialysis. Conservative therapy also alleviates total base deficit. "Fixed acid" excess was present in only three of the ten patients. However, of the seven patients having no increase in the total serum non-buffer anion concentration, six had hypochloremia which offset the increase in the non-chloride "fixed acids." Hemodialysis results in correction of the hypochloremia and reduction of non-chloride "fixed acids." Conservative treatment on the other hand usually results in further hypochloremia and does not reduce significantly the level of other "fixed acids."

The development of respiratory alkalosis in three patients during correction of the metabolic acidosis by dialysis is similar to that resulting from conservative therapy, utilizing sodium lactate, as reported by Yeomans and Stueck (Case I) (10). The production of respiratory alkalosis by alkali therapy of metabolic acidosis is not confined to the treatment of renal acidosis but has been noted previously during the treatment of diabetic acidosis. This substitution of a primary respiratory alkalosis for a primary metabolic acidosis has been elaborated on by Singer and Hastings (9). Wallace has also discussed this lag in respiratory adjustment during base administration and has pointed out that there is independence of the acid-base balance of intra- and extracellular fluids and that rapid alkali therapy has a minimal effect on intracellular pH (11). The rapidity of change of the serum bicarbonate concentration during dialysis suggests that the extracellular fluid rather than total body water is equilibrating with the dialysate bath. This is in accord with the observation of Rosenbaum that intravenous bicarbonate appeared to be limited in its distribution to the extracellular fluid (12). Wallace and Hastings (13) also found that the bicarbonate concentrations in muscle cells is not changed by elevation of the serum bicarbonate if the P_{CO_2} is unchanged. It would appear that during dialysis, although there is a prompt change in the extracellular bicarbonate concentration and pH, there is little change in the intracellular bicarbonate and pH, with the result that there is little change in the metabolism within the respiratory center and, therefore, no stimulus for alternation of the respiratory exchange and P_{CO_2}. A secondary respiratory alkalosis which is present at the onset of dialysis can by this means persist and be the sole disturbance of the acid-base balance at the end of dialysis when the primary metabolic acidosis has been corrected.

The rapidity of the onset of clinical improvement following dialysis varies according to the disease state present. The removal by dialysis of a toxic ion such as bromide results in clinical improvement within a few hours (14). The bromide ion is present primarily in the extracellular fluid and can be readily removed by dialysis. Patients having the uremic syndrome, however, do not show such a prompt clinical response. Those with acute renal insufficiency usually improve clinically within 24 hours, while those with chronic renal insufficiency may not improve for 48 or 72 hours. It would seem doubtful in these cases of chronic renal insufficiency that the efficacy of hemodialysis is directly due to the removal of a toxic metabolite which has accumulated primarily in the extracellular fluid or which is easily diffusible between the intracellular and extracellular fluids. Likewise, the delayed clinical improvement cannot be directly correlated with the correction of the lowered pH, decreased buffer base, hypochloremia, or "fixed acid" excess. All of these change rapidly during dialysis. However, it is possible that a delay in the readjustment of the intracellular pH and cellular metabolism may explain the delay following dialysis before clinical improvement is shown by chronically ill, severely uremic patients.

SUMMARY

The changes in the acid-base balance during hemodialysis have been studied in ten uremic patients. Nine of the patients were acidotic with a lowered serum pH. All ten patients had a primary metabolic acidosis with a diminished whole blood buffer base concentration due to a decreased concentration of both the protein and bicarbonate anions. Eight patients had a diminished total serum base concentration, and three had "fixed acid" excess. Five patients had a secondary respiratory alkalosis.

Hemodialysis alleviated the acidosis; increased the buffer base concentration, primarily by increasing the bicarbonate fraction; increased the total serum base concentration, and removed nonchloride "fixed acids," but at the same time, corrected hypochloremia. The respiratory alkalosis, when present, persisted unchanged throughout dialysis.

The relationships between these changes in the acid-base balance and clinical improvement following hemodialysis have been discussed.

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THE EFFECT OF AN IMPERMEABLE VAPOR BARRIER ON ELECTROLYTE AND NITROGEN CONCENTRATIONS IN SWEAT [1]

By CHARLES R. KLEEMAN,[2] DAVID E. BASS, AND MURRAY QUINN [3]

(From the Department of the Army, Quartermaster Climatic Research Laboratory, Lawrence, Massachusetts)

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When environmental temperatures are high, 80 to 90 per cent of the total water, sodium, and chloride, and 25 to 50 per cent of the losses of potassium, calcium, magnesium, and nitrogen may occur in the sweat (1, 2). Failure to measure these losses may lead to serious errors in balance studies. Although dermal water loss can be quite accurately measured in both acute and prolonged studies, this is not true of sweat electrolytes and nitrogen. Even in metabolic balance studies where the subject's daily activities are rigidly controlled, the accurate collection of all body sweat solutes by repeated body washings presents obvious difficulties. Conn and Louis (3) and Johnson, Pitts, and Consolazio (4) have assumed that the dermal losses are equal to the intake—(urine plus fecal losses). This method, however, disregards any daily positive or negative balances of these substances which may occur. The simplest method involves the collection of a local sweat sample, measurement of the solute concentration, and determination of the total solute content from the concentration and the total sweat loss. This method assumes that the local sample is representative of the total body sweat and that the technique of collection does not alter the true solute concentrations. Previous investigations (5, 6, 7, 8) and data from this Laboratory (7) indicate that neither of these assumptions is correct. In general, previous studies (5, 6, 7, 8, 9) indicate that the chloride, nitrogen, and lactic acid concentrations of arm sweat collected under an impermeable barrier, are higher than the concentrations of these solutes in total body sweat. Mickelsen and Keys (6) have shown that the concentrations of chloride ion, urea, and lactic acid are different in sweat samples collected under identical conditions from different body areas. They also noted that the concentration of chloride ion in hand sweat collected in a rubber glove is 38 to 72 per cent higher than the mean concentration of chloride ion in sweat collected from the entire body by repeated washings.

In contrast, Ladell (5) found surprisingly good agreement between the loss of chloride in sweat calculated from arm bag concentrations and sweat rate and the value obtained from total body washings.

In view of the conflicting results of previous investigators, the present study was undertaken to reevaluate the effect of an impermeable barrier, such as a plastic arm bag on the sodium, potassium, chloride, and total nitrogen concentrations of thermal sweat and to determine whether the concentration of solutes in arm bag sweat could be used to calculate total dermal electrolyte and nitrogen losses.

METHODS

Twenty-seven experiments were performed on 17 men. Although the precise state of acclimatization to heat was not known in these subjects, one group of experiments was performed during the fall (Group A) and the other (Group B) during the summer. Each experiment consisted of a one- to two-hour exposure at 120° F. with a relative humidity of 28 to 30 per cent. In an effort to prevent the loss of any body sweat by means other than evaporation, the men were clothed in light cotton pajama-type drawers, the lower end being tucked into long woolen socks, covered over with polyethylene foot bags inside of combat boots. Large cotton bath towels, folded in strip fashion, were tied around the chest and the unin-closed upper arm. Each subject carried an additional towel in his hand for wiping off his face and neck. In all subjects, sweat was collected during the entire heat exposure from one arm in polyethylene bags which were sealed at the level of the insertion of the deltoid muscle. During the period of sweat collection, the subject alternated periods of walking on a horizontal motor-

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[2] Present address: Department of Internal Medicine, Yale University School of Medicine, New Haven, Conn.

[3] 1st Lieutenant, Medical Corps.

driven treadmill at four mph with rest. The alternate periods were fifteen to twenty minutes in duration.

The experiments were divided into two groups. In Group A (twelve experiments), the subjects were exposed in the nude to the heat for a 15- to 30-minute period to initiate sweating and "flush" the lumens of the sweat glands and the surface of the skin. Following this brief heat exposure each subject showered. After this he was rinsed with three to five liters of distilled water, dried, weighed to ± 10 grams and reentered the heat, appropriately dressed, with the polyethylene bag on one arm. In Group B (15 experiments), no preliminary exposure to heat was employed. In this group, therefore, sweat collections were begun without prior "flushing" of sweat gland lumens. The remainder of the procedure was similar to that employed for Group A.

The arm bag was removed at the end of the exposure to heat in all 27 experiments. The sweat was mixed thoroughly in the bag by shaking, filtered into polyethylene bottles, and the volume measured. The solutes of the total body sweat were recovered by washing each man as he stood or kneeled in a large porcelain bathtub with 15 to 20 liters of distilled or tap water.[4] The subjects were then dried and reweighed. The plastic bag, all towels, and clothing utilized to collect sweat during the heat exposure were added to the wash water in which they were thoroughly rinsed.

The final volume of the wash water was determined by adding 500 mgm. of antipyrine and calculating the volume from the dilution of antipyrine. Electrolyte and nitrogen concentrations were determined in the arm bag sweat and in the final wash water. Sodium and potassium were determined on a Beckman flame photometer, correction being made in the standards for mutual excitation of sodium and potassium. Chloride was determined by the Schales and Schales method (10), or a modification of the Volhard silver nitrate procedure (11), and total nitrogen by the Conway microdiffusion method (12).

[4] The tap water was analyzed for its content of sodium, chloride, potassium, and nitrogen.

Method of calculation

a) Sweat loss (Kg.) = Weight − Respiratory H_2O loss.[5]
b) Arm bag content of each solute = Volume of sweat collected in the arm bag × concentration of the individual solute in arm bag sweat.
c) Calculated solute loss = Sweat loss (Kg.) × concentration of the individual solute in arm bag sweat.
d) Solute recovered from the total body = (Volume of wash water × concentration of the solute in the wash water) + Arm bag content of each solute (b).
e) Concentration of the measured solute in total body sweat = Solute recovered (d)/Sweat loss Kg. (a).

[5] Respiratory water loss was calculated by determining the ventilation rate of the subjects while walking on the treadmill and at rest; the rates were corrected to body temperature and the expired air was assumed to be saturated with water vapor at 37° C. This assumption introduces a small error since expired air need not be saturated.

RESULTS

The results are summarized in Tables I and II.

Sweat rate. No significant correlations were noted between sweat rate and the arm or total body concentrations of the various solutes studied. Previous investigators (4, 7, 13, 14, 15), however, have demonstrated close correlations between sweat

TABLE I

Comparison of sweat concentrations from arm and total body (Group A)

No.	Sweat rate	Na^+ (1)*	Na^+ (2)*	K^+ (1)	K^+ (2)	Cl^- (1)	Cl^- (2)	N (1)	N (2)
	cc./hr.	*mEq./L.*	*mEq./L.*	*mEq./L.*	*mEq./L.*	*mEq./L.*	*mEq./L.*	*mgm. %*	*mgm. %*
1	1,275	65.9	51.2	4.6	3.1	58.3	43.2	41.3	28.3
2	752	103.2	75.0	9.0	4.9	86.3	65.3	56.5	35.0
3	554	114.0	72.8	15.8	6.7	92.5	59.5	—	—
4	917	115.4	77.3	6.4	4.1	101.6	68.5	42.1	31.5
5	536	72.8	56.7	5.9	5.1	64.9	49.4	50.8	41.1
6	1,235	45.4	45.2	6.1	4.7	39.1	37.0	45.3	39.9
7	1,168	36.1	40.5	5.7	4.0	29.9	30.0	55.3	31.1
8	873	29.4	32.3	5.9	4.9	24.6	22.0	43.0	37.1
9	1,204	61.0	52.9	6.1	5.0	51.9	44.9	39.3	37.2
10	1,316	73.5	63.8	7.1	5.8	63.7	54.9	40.5	27.1
11	2,036	61.6	51.2	4.2	3.1	53.7	41.9	31.0	24.4
12	822	94.7	86.9	6.4	5.3	81.8	70.1	54.6	40.1
	Mean	72.7	58.8	6.9	4.7	62.4	48.9	45.4	33.8
	S.D.	±28.9	±16.5	±3.0	±1.0	±24.5	±15.3	±8.0	±5.7
	Significance of differences	$P < .01$		$P < .01$		$P < .01$		$P < .001$	

* (1) = Concentration of a given solute in arm bag sweat.
(2) = Concentration of a given solute in sweat recovered from the entire body.

TABLE II

Comparison of sweat concentrations from arm and total body (Group B)

No.	Sweat rate	Na^+ (1)*	Na^+ (2)*	K^+ (1)	K^+ (2)	Cl^- (1)	Cl^- (2)	N (1)	N (2)
	cc./hr.	*mEq./L.*	*mEq./L.*	*mEq./L.*	*mEq./L.*	*mEq./L.*	*mEq./L.*	*mgm. %*	*mgm. %*
1	1,058	72.2	54.8	8.7	6.3	70.1	59.8	65.4	37.5
2	912	81.6	60.0	9.4	6.4	79.6	64.3	63.2	29.8
3	914	29.4	21.4	7.3	5.2	25.0	24.3	49.0	26.1
4	1,315	59.0	34.3	5.9	5.2	54.4	49.0	38.9	26.2
5	1,249	72.4	52.4	11.1	5.0	70.3	55.2	49.3	34.0
6	978	59.0	49.4	7.8	4.4	56.9	54.2	43.5	23.5
7	913	74.8	44.9	12.4	4.6	69.8	52.6	115.8	29.1
8	1,225	94.6	49.5	15.0	6.3	88.1	57.1	—	—
9	1,220	42.4	33.5	5.8	4.0	41.4	44.6	55.5	31.6
10	850	68.0	42.3	9.7	5.1	67.9	50.7	57.6	20.0
11	1,094	60.5	42.1	8.2	5.8	62.4	44.0	29.4	17.2
12	1,192	58.9	43.6	7.8	5.6	60.7	49.1	48.0	32.5
13	522	32.9	21.1	7.2	3.5	24.6	18.1	74.0	25.8
14	494	19.0	13.8	8.7	4.4	12.3	12.9	81.3	18.6
15	549	49.2	37.9	6.9	4.3	41.7	33.2	53.8	22.4
	Mean	58.6	40.1	8.8	5.1	55.0	44.6	58.9	26.7
	S.D.	±20.7	±13.3	±2.4	±.9	±23.4	±15.5	±21.3	±5.9
	Significance of differences	$P < .001$		$P < .001$		$P < .001$		$P < .001$	

* See footnote, Table I.

rates and sodium and chloride concentrations in any given individual under different states of acclimatization and at different environmental temperatures. In the present study similar correlations might have been noted if sweat rates had been corrected for surface area, and if each subject had been compared with himself at different environmental temperatures and different sweat rates.

Sodium and chloride. The mean sodium concentration of arm bag sweat was 72.7 mEq. per L. for

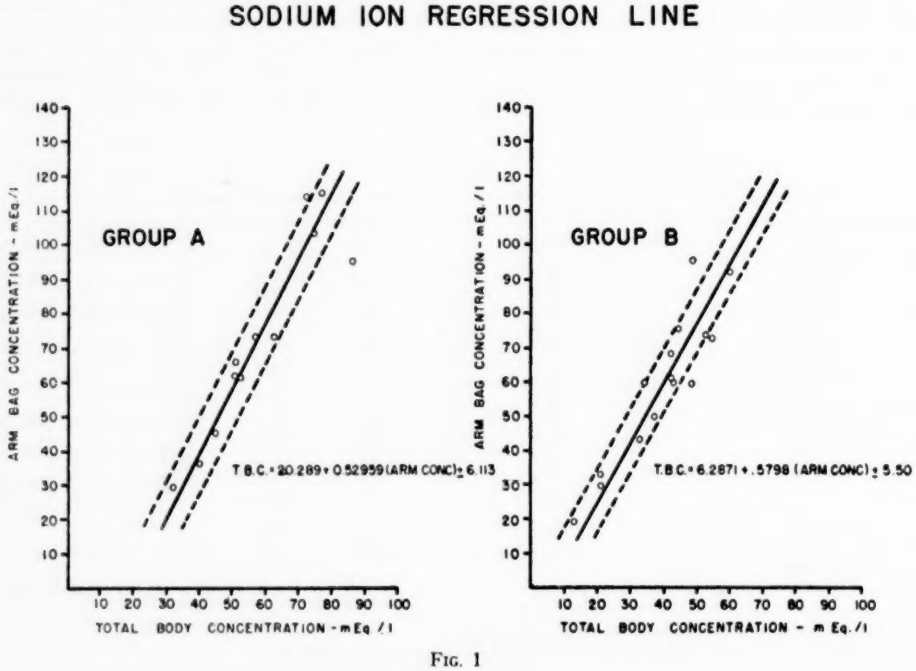
SODIUM ION REGRESSION LINE
GROUP A
GROUP B
ARM BAG CONCENTRATION - mEq./l
TOTAL BODY CONCENTRATION - mEq./l
T.B.C. = 20.289 + 0.52959 (ARM CONC) ± 6.113
T.B.C. = 6.2871 + .5798 (ARM CONC) ± 5.50

FIG. 1

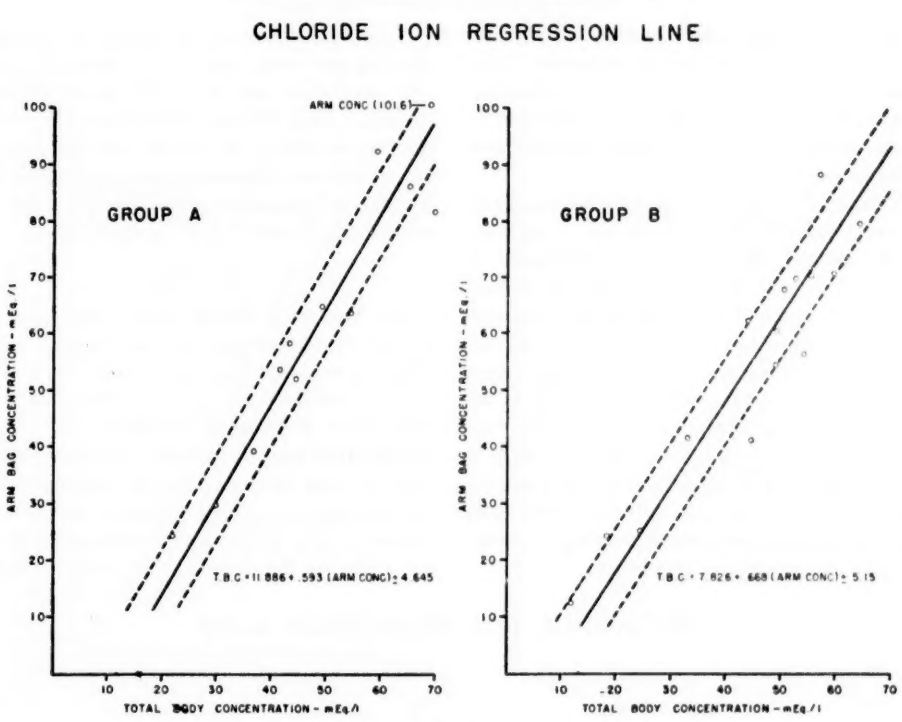

FIG. 2

Group A and 58.6 mEq. per L. for Group B. The mean sodium concentration of total body sweat was 58.8 mEq. per L. for Group A and 40.1 mEq. per L. for Group B. The differences (between Groups A and B) in total body and arm bag sweat concentrations might have resulted in part from the difference in the preliminary "flushing" procedure, as well as the element of heat acclimatization. Group B experiments were performed during the summer, and Group A experiments were performed during the late fall (16). In both groups the mean total body concentration was appreciably lower than the arm bag concentration. These differences were highly significant. In general, the chloride concentrations of the sweat in both groups followed a pattern similar to that observed for sodium. However, it should be noted (Tables I and II) that the sodium concentration of arm bag sweat exceeded the chloride concentration in every instance, but the chloride concentration of total body sweat exceeded the sodium concentration of total body sweat in twelve of the 27 experiments. From the data of Tables I and II it appeared that the lower the arm bag sweat concentrations of sodium and chloride the closer was the agreement with total body concentrations. Therefore, regression equations were formulated relating the concentrations of sodium and chloride in total body sweat to those in arm bag sweat (Figures 1 and 2). These equations and lines validated the above observations. There appeared to be very little difference between Groups A and B with respect to the chloride slope; however, the slope obtained for sodium in Group A demonstrated closer agreement than Group B between the calculated and actual recovery of sodium.

Potassium. The mean potassium concentration in arm bag sweat for Groups A and B was 6.93 mEq. per L. and 8.77 mEq. per L., respectively, whereas the mean concentration in total body sweat for these groups was 4.71 mEq. per L. and 5.08 mEq. per L., respectively. Again these differences between total body and arm bag concentrations were highly significant. It was noted in both groups (Tables I and II) that the potassium

concentration in arm bag sweat in many cases greatly exceeded normal plasma potassium levels, whereas in only four instances was the concentration in total body sweat as high as 6 mEq. per L. The remainder (82 per cent) were all in the normal plasma range.

The mean difference between total body and arm bag sweat concentrations for Group A was appreciably smaller than the mean difference in Group B. In contrast to the findings on sodium and chloride, there was no apparent improved agreement between arm and body sweat concentrations of potassium with lower arm bag values (Figure 3). In general, regardless of the variation in arm bag concentrations, the total body concentrations fell within a narrow range. Group A differed from Group B not only in having a smaller average difference between arm and total body sweat concentration, but also in showing a smaller spread of arm bag values (Figure 3).

Nitrogen. The concentrations of nitrogen in arm bag and total body sweat followed a pattern very similar to that previously described for potassium. They did not demonstrate the arm-body correlations shown by sodium and chloride (Figure 4) and the differences between Groups A and B noted for potassium were also noted for nitrogen (Tables I and II and Figure 4).

DISCUSSION

The two main factors which may have given rise to the differences in the concentrations of solute in arm and total body sweat were (1) errors in technique and (2) unknown and/or uncontrollable physiologic variables. In recovering solutes from total body sweat, loss of liquid sweat from the skin surface during the exposure to heat, and incomplete washing constitute major sources of error. The former was minimized in the present study by the use of a low relative humidity

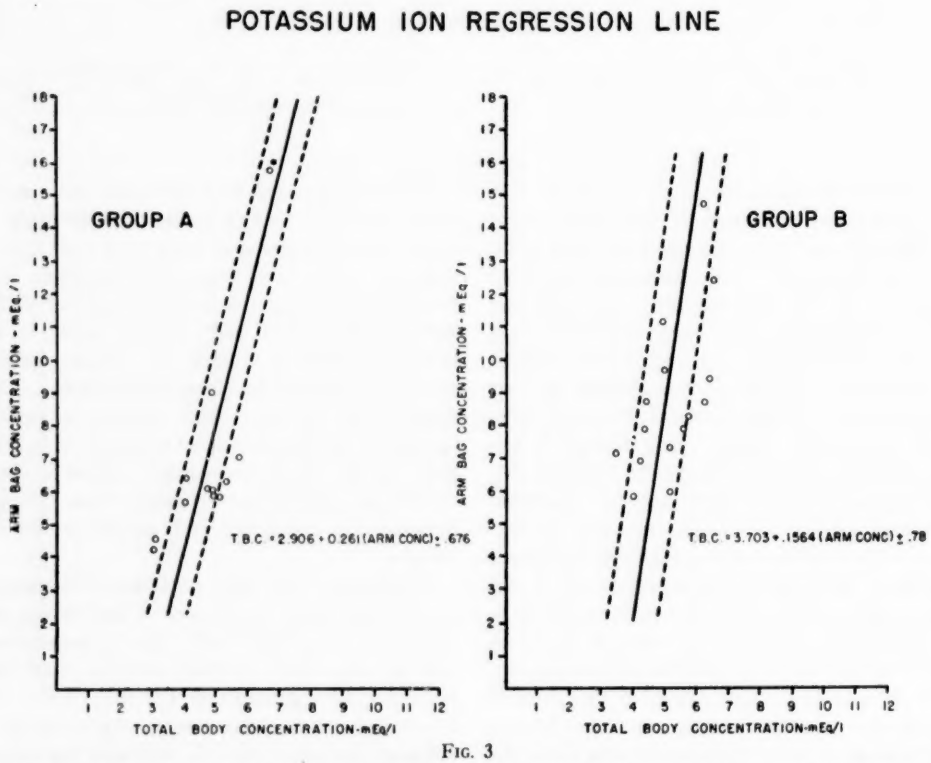
POTASSIUM ION REGRESSION LINE
GROUP A
GROUP B
ARM BAG CONCENTRATION - mEq./l
TOTAL BODY CONCENTRATION-mEq/l
T.B.C. = 2.906 + 0.261 (ARM CONC) ± .676
T.B.C. = 3.703 + .1564 (ARM CONC) ± .78

FIG. 3

This subject with the highest arm bag potassium concentration in Group A had a surprisingly low arm sweat volume. This made thorough mixing of his arm bag sweat difficult.

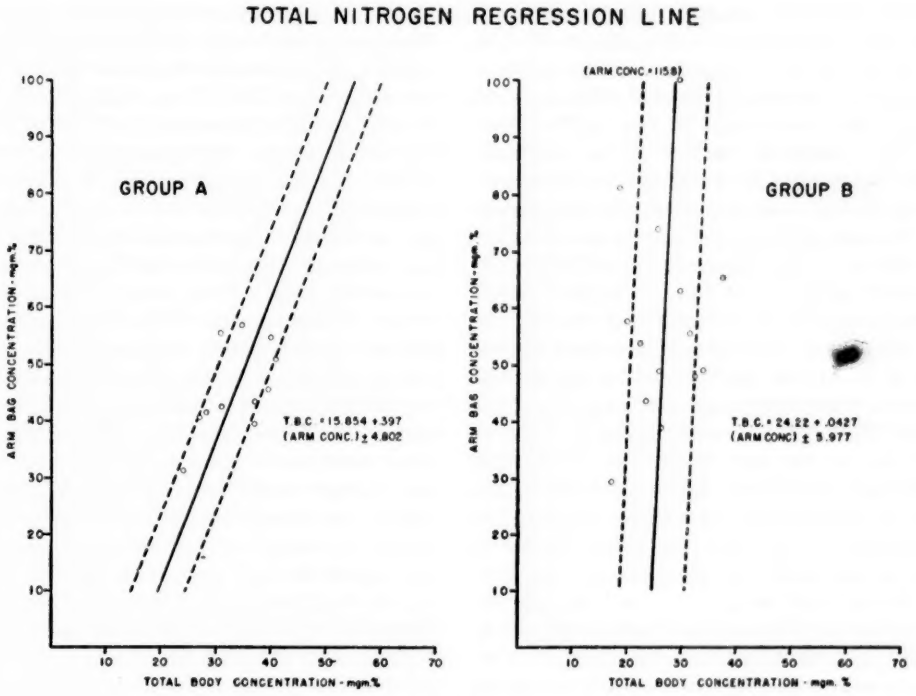
TOTAL NITROGEN REGRESSION LINE
GROUP A
GROUP B
ARM BAG CONCENTRATION - mgm.%
TOTAL BODY CONCENTRATION - mgm.%
T.B.C. = 15.854 + .397 (ARM CONC.) ± 4.802
T.B.C. = 24.22 + .0427 (ARM CONC) ± 5.977
(ARM CONC = 1158)

FIG. 4

which enhanced evaporation and by the use of towels and underwear to soak up the sweat. In this study 15 to 20 liters of water were used in washing each man. This was considerably more water than has been used in previous studies of this type (5, 7, 8, 15) in which complete recovery of all sweat solute on the body surface has been demonstrated. Errors in determining the volume of wash water were minimized by measuring the dilution of known amounts of antipyrine added to the wash water. Errors in solute concentration in arm bag sweat could occur if, (1) the sweat collected in the dependent part of the bag was not thoroughly mixed with the water vapor which had condensed along the walls of the plastic bag, and (2) if this condensed water vapor did not mix with the more concentrated sweat on the skin surface. These errors were minimized by thorough mixing of the sweat in the bag after it was removed from the arm and by constant movement of the arm in contact with the inner surface of the bag while the subjects walked the treadmill. In calculating sensible sweat losses by difference in weight, correction should be made for other avenues of weight change. Since no fluids were taken by mouth and no urine or feces were passed between weighings, no correction was necessary for these sources of weight change. Insensible water loss [6] was a necessary correction. Calculated respiratory water loss averaged about 70 grams per hour in this study, and at the high sweat rates noted, even large percentage errors in respiratory water loss would have altered the calculated sweat losses very little. Insensible water loss through the skin theoretically stops when the skin surface is covered with moisture (16). However, the low humidity present in this study is conducive to rapid evaporative losses, leading to concentration of the sweat solute on the skin surface. This may have enhanced osmotic water loss through the skin. This loss cannot be quantitated, but probably represents negligible volume.

The concentrations of the solutes in total body sweat determined in this study were in the ranges

[6] Insensible water loss is defined as a dermal or pulmonary loss of water vapor without solute.

noted by numerous other investigators who collected sweat solutes in a similar manner (5, 6, 8, 17, 18, 19, 20, 21). Inspection of the data emphasizes the large dermal losses of electrolytes and nitrogen that may occur during high rates of sweating. Metabolic balance studies conducted in this Laboratory (2) during which subjects were exposed to high temperatures for 24-hour periods have demonstrated maximal sweat losses of sodium and chloride of 400 mEq., potassium 60 mEq., and nitrogen 5 grams per 24 hours. Under conditions similar to those of the present study the large errors involved in utilizing arm bag sweat concentrations of sodium, chloride, potassium, and nitrogen in determining total body sweat losses and metabolic balances are readily apparent.

Because of the ease of collection of arm bag sweat it was hoped that some constant relationship could be demonstrated between the concentration of solutes in arm and total body sweat. Under the experimental conditions used in both Groups A and B, total body sweat sodium and chloride concentrations could be fairly well predicted from the arm bag concentrations using the formulated regression equations (Figures 1 and 2). From the standard errors of estimate of these equations, the accuracy of prediction is, however, not very high.

Examination of the data of various investigators as summarized by Dill (13) showed that the lower the concentration of chloride in hand sweat, the more closely did it agree with the concentration of chloride in the total body sweat. The subjects in whom Ladell (5) found good correlations between recovered and calculated sweat chloride loss were probably acclimatized to heat and they all showed relatively low chloride concentrations in the arm bag sweat. In the present study, it is precisely in this group that the closest correlation between chloride concentrations in arm and total body sweat were noted.

In a recent study, van Heyningen and Weiner (8) demonstrated that the arm bag chloride, urea, and lactate concentrations always exceeded the concentrations of these substances in total body sweat. They, however, collected their arm bag sweat by draining it off at intervals from the bottom of the bag, a process that probably would lead to excessive concentration of the sweat as a result of water vapor condensation on the sides of the bag. The lowest arm bag chloride concentration given in their study was 81 mEq. per L.

It is difficult to explain the closer agreement between arm bag and total body sweat concentrations of sodium and chloride associated with lower arm bag concentrations. Sodium and chloride concentrations of sweat collected under an impermeable barrier (8, 22) are influenced by local factors such as the skin temperature and the vapor pressure under the barrier, and systemic factors such as adrenal cortical activity, state of acclimatization, dietary salt content, etc. If the lower sodium and chloride concentrations were an indication of greater systemic influence on sweat gland activity, this influence would exceed that of the local environmental factors created under the bag. A closer correlation between arm bag and total body concentration would then be noted. A closer correlation at lower concentrations would also be noted if a constant volume of sweat water in the bag repenetrated the skin. The absolute change in water to solute relationship would have a greater effect the higher the original solute concentration in the sweat. Although the present study offered no means of assessing the contribution of these factors, our failure to obtain correlations for arm and total body nitrogen and potassium similar to those found for sodium and chloride suggested that there was some selective action on the sodium and the chloride ions.

In general, the total body potassium concentration fell within the narrow range of approximately 4 to 6 mEq. per L., whereas the concentration in the arm bag sweat showed wide variation up to levels as high as 15 mEq. per L. This lack of any correlation between arm bag and total body concentrations makes only gross approximation of the correct skin losses possible.

The differences between Groups A and B, relative to potassium and nitrogen, should be emphasized. Not only were the differences between the arm bag and total body sweat concentration smaller in Group A ("flushed" group) than in Group B, but Group A showed a smaller spread of arm bag values. These differences between groups might be best explained by the contribution to the sweat in Group B of high potassium and nitrogen containing material that was removed in Group A by the "flushing" period before initiating sweat col-

lections. This material could be either stagnant or concentrated solutes in the sweat gland lumen or secretions from the sebaceous glands. Cutaneous desquamation is probably of minor importance because the phosphorus concentration in sweat is negligible regardless of the rate of sweating, or the absence of a "flushing" period (1, 2, 19, 23, 24, 25). It would be much higher if a large cellular contribution were present. Furthermore, all sweats were filtered immediately after being collected.

Berenson and Burch (26) recently demonstrated that the highest sweat potassium concentrations were seen in the earliest period of serial sweat collections, and the concentrations then fell to a relatively constant value. The reverse was seen for sodium and chloride concentration. Many years ago Whitehouse, Hancock, and Haldane (16, 25, 27, 28) demonstrated that the lower the sweat rate (below 300 cc. per hr.), the greater the potassium to chloride ratio and the higher the organic solids to ashed solids. The contamination of sweat by substances high in potassium best explained these findings. The results of this study and those reported in the literature emphasize the importance of this preliminary "flushing" period before attempting to obtain the "true" sweat concentration of potassium and nitrogen.

Although sweat solute concentrations vary greatly in different body areas, there is no evidence that the arm sweat concentrations of the measured solutes are higher than the concentration of these solutes from other skin areas collected in a similar manner (4, 5, 6, 14, 26). It would appear that the environment created under the arm bag led to the differences noted between arm and total body sweat solute concentration.

Ladell found that when sweat was collected from both arms under similar conditions, the chloride concentrations and sweat rates were equal; however, when he compared the inclosed with the uninclosed arm, he found that both the sweat rates and chloride concentrations of the inclosed arm were higher. He, as well as others (5, 7, 15), noted that the skin temperature of the inclosed arm was always higher than the uninclosed arm or the rest of the skin surface. The positive correlation between skin temperature, sweat sodium, and chloride concentrations, and sweat rate has been stressed in previous studies (4, 5, 7, 15, 29).

Although the skin temperature is an important factor in the differences between arm bag and total body sweat concentrations, it is not adequate to explain all the findings. Weiner and van Heyningen (21) found no change in urea concentration with increasing skin temperatures, and lower skin temperatures with lower sweat rates are probably associated with higher potassium concentrations (2, 26, 27, 28). Van Heyningen and Weiner (8) hypothesized that if a single factor within the arm bag affected all the solutes equally, then their concentrations would all change proportionally; therefore, the ratio of the total body concentration of a given solute to the total body concentration of any other solute should equal the ratio of the arm bag concentrations of these same solutes, *i.e.*, $(N)_a/(Cl^-)_a$ should equal $(N)_b/(Cl^-)_b$. Their correlation coefficients for urea, chloride, and lactate were very high. They concluded that the arm bag environment affects primarily the movement of sweat water, the latter undergoing repenetration or reabsorption through the skin surface. This would, of course, lead to equal degrees of concentration of all sweat solutes. Repenetration of sweat water through the intact skin probably occurs (16, 27, 30) without solutes (22). This is enhanced by increases in skin temperature and skin blood flow (30) as found within the arm bag.

In an effort to substantiate the findings of van Heyningen and Weiner (8), the arm and body concentrations of the various solutes were correlated in a similar manner. The data are presented in Table III. Many correlations comparable to those of van Heyningen and Weiner were obtained. The sodium to chloride correlations and the potassium to nitrogen correlations were not significant. It would appear that movements of water without solute were important, but other factors must contribute to the difference between the concentration of solutes in arm bag and total body sweat.

Finally, the inclusion of hand or palmar sweat in the collection of arm bag sweat may be of considerable importance in causing differences between the arm bag and total body concentrations found. Palmer and plantar sweat have characteristics that differ markedly from general body sweat. Randall and Hertzman (31, 32) and Weiner (33)

TABLE III

Correlation of solute concentration ratios in the arm sweat (a) with solute concentration ratios in the body sweat (b)

Solutes correlated	Correlation coefficients	Level of significance
Group A		
$\frac{Cl^-_a}{Na^+_a}$ vs. $\frac{Cl^-_b}{Na^+_b}$	.515	Not significant
$\frac{K^+_a}{Na^+_a}$ vs. $\frac{K^+_b}{Na^+_b}$	.918	$P < .01$
$\frac{N_a}{Na^+_a}$ vs. $\frac{N_b}{Na^+_b}$	.838	$P < .01$
$\frac{N_a}{Cl^-_a}$ vs. $\frac{N_b}{Cl^-_b}$	.866	$P < .01$
$\frac{N_a}{K^+_a}$ vs. $\frac{N_b}{K^+_b}$	.603	$P < .05$
$\frac{K^+_a}{Cl^-_a}$ vs. $\frac{K^+_b}{Cl^-_b}$	.921	$P < .01$
Group B		
$\frac{Cl^-_a}{Na^+_a}$ vs. $\frac{Cl^-_b}{Na^+_b}$	.509	$P = .05$
$\frac{K^+_a}{Na^+_a}$ vs. $\frac{K^+_b}{Na^+_b}$	.905	$P < .01$
$\frac{N_a}{Na^+_a}$ vs. $\frac{N_b}{Na^+_b}$	.812	$P < .01$
$\frac{N_a}{Cl^-_a}$ vs. $\frac{N_b}{Cl^-_b}$	.851	$P < .01$
$\frac{N_a}{K^+_a}$ vs. $\frac{N_b}{K^+_b}$	.520	$P < .05$
$\frac{K^+_a}{Cl^-_a}$ vs. $\frac{K^+_b}{Cl^-_b}$	.952	$P < .01$

have shown that not only is sweating continuous at cool temperature on the palms and soles, but palm and sole sweating does not appear to be related to environmental temperatures. In chemical analysis of palmar sweat collected by cannulation, Lobitz and Mason (34) noted chloride concentrations ranging from 100 to 300 mEq. per L. and urea concentrations from 68 to 275 mgm. per 100 cc. of sweat. Since our collections included sweat from the palm, we cannot at this time assess its contribution to the results observed.

SUMMARY AND CONCLUSIONS

In 27 experiments on 17 healthy, young, white men the sodium, potassium, chloride, and nitrogen concentrations of total body sweat were compared with their respective concentrations in arm bag sweat. In twelve experiments (Group A) the sweat collections were preceded by a short heat exposure to "flush" the sweat gland lumens and skin. In Group B (15 experiments), no such "flushing" period was used.

The nitrogen and potassium concentrations of arm bag sweat always exceeded the concentrations of these solutes in total body sweat. This difference was exaggerated in the Group B experiments. In neither group could predictable correlations be found between total body and arm bag nitrogen and potassium sweat concentrations. However, in almost all cases the total body sweat concentrations of nitrogen and potassium fell within narrow range of 20 to 40 mgm. per cent and 4 to 6 mEq. per L., respectively. The lower the sodium and chloride concentrations were in arm bag sweat, the closer was the agreement with the total body sweat concentrations of these electrolytes.

Regression equations were formulated from these data and the usefulness of these equations for the prediction of total body sweat concentrations of sodium and chloride were discussed. An attempt was made to delineate the technical sources of error and the physiologic variables accounting for the differences between arm bag and total body concentrations noted in this study.

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TRACER EXPERIMENTS WITH I^{131} LABELED HUMAN SERUM ALBUMIN: DISTRIBUTION AND DEGRADATION STUDIES

By SOLOMON A. BERSON, ROSALYN S. YALOW, SIDNEY S. SCHREIBER, AND JOSEPH POST

(From the Radioisotope Unit and the General Medical Research Laboratory of the Veterans Administration Hospital, Bronx, New York, N. Y.)

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The distribution and metabolism of serum proteins in human subjects have been investigated recently by means of tracers labeled *in vitro* with I^{131} or biosynthesized with S^{35} or N^{15}. A study by London (1) indicated a half time of about 20 days for N^{15} labeled serum albumin turnover in a normal man and woman. In two normal subjects an average half time value of 17.1 days for serum albumin was obtained by Volwiler, Fremont-Smith, and MacMartin (2) following the oral administration of S^{35} tagged dl-cystine. Sterling (3), however, utilizing the disappearance rate of injected I^{131} tagged human serum albumin, reported a mean half time of 10.5 ± 1.5 days in 21 normal medical students, the longest being 13.4 days. Similarly, marked differences in the average half times of gamma globulin as determined by I^{131} labeling and S^{35} biosynthesis have been reported. Values of 7.0 to 8.8 days (4) and 7.8 days (5) have been found by the former method as contrasted with 27.0 days by the latter (2).

These discrepancies merit further investigation into the validity of the methods employed, especially in view of the increasing application of I^{131} tagged proteins to the study of turnover rates in health and disease. In the present study the fate of intravenously administered I^{131} labeled human serum albumin has been followed over periods of time considerably longer than have heretofore been reported. The results emphasize the difficulties in interpretation of data and highlight the shortcomings of this method of analysis. They also aid in resolving the discrepancies noted above.

METHODS

Iodination of albumin. Lots of albumin iodinated by three different techniques were utilized. Two methods were employed in the laboratory and several batches prepared by each were studied.[1] The final products contained an average of 1 to 2 atoms of iodine per molecule of protein except in one case. Method A was identical with that reported by Pressman and Eisen (6). When relatively large amounts of I^{131} solution[2] were used, only 8 to 10 per cent of the radioactive iodine became protein bound, probably owing to the reducing effect on elemental iodine of the acid sodium sulfite accompanying the I^{131}. In order to increase the efficiency of radioactive labeling and to decrease radiation exposure during preparation, method B (7) was used. In this case, the elemental iodine was similarly formed from a mixture of stable and radioactive iodide by nitrous acid but it was protected from reduction to iodide by its removal from the mixture into a chloroform phase. Since the iodine-protein combination occurs rapidly the chloroform-iodine solution was added slowly to the continually stirred solution of albumin in order to obtain optimal random iodination. By this procedure, 25 to 35 per cent of the I^{131} became protein bound. As is indicated below, there has been no reason to suspect an adverse effect of the chloroform on the albumin, the lots prepared by this method proving the most satisfactory of all those studied. In many experiments, labeled albumin prepared by Abbott Laboratories and specified to contain 1 to 2 I atoms per molecule protein was used. Although, before shipment, this material had been passed through resin columns designed to absorb residual iodide and iodate, usually about 3 to 5 per cent and occasionally as much as 8 per cent was found to pass a Visking cellulose membrane upon receipt in the laboratory. Therefore, all lots of iodinated albumin utilized were dialyzed in the cold against frequently changed normal saline solutions for about 48 hours. Lots prepared in this laboratory were passed through Seitz filters and tested for sterility by culture before use. Cold 10 per cent trichloracetic acid precipitated 98 per cent or more of the radioactivity in the final products.

Ultracentrifugal and electrophoretic analysis of iodinated proteins. Sample lots of labeled albumin were subjected to ultracentrifugal[3] and electrophoretic analysis. The electrophoretic patterns[4] showed a single peaked symmetrical curve identical to that obtained with "native"

[1] We are indebted to the American National Red Cross for supplies of "normal" human serum albumin manufactured and packed by the Armour Laboratories, Ft. Worth, Texas.

[2] Obtained from Oak Ridge National Laboratory.

[3] Thanks are due to Dr. Kurt G. Stern, Brooklyn Polytechnic Institute, for the ultracentrifuge analyses.

[4] 0.5 per cent solution, phosphate buffer, pH 7.4.

albumin. Mixtures composed of equal parts of iodinated and native albumin also showed a single peak. Similarly, ultracentrifugal analysis usually failed to detect inhomogeneity or differences between labeled and native albumin, even when the biological behaviour of the labeled albumin was such as to suggest altered protein, at least in part.

Assay of radioactivity. Several counting systems were simultaneously employed, and each was standardized with respect to the others. *In vivo* assays were performed with a bismuth walled Geiger counter replaced daily in an identical position with respect to that part of the body being assayed. At 5 and 9 inches respectively, this counter had a sensitivity of approximately 250 and 100 counts per minute per μc of I^{131}, depending on the *in vivo* source of radioactivity, above a background of 60 counts per minute. Because of a wide window in the counter shield exposing the entire sensitive region of the tube (five inches $\times$ one inch), a fair sized volume of even such large organs as the liver could be "seen" by the counter at these distances. The rates of disappearance of radioactivity from sites of injection of tagged albumin were determined by methods previously described (8, 9). In some cases a scintillation counter was used.

Radioactivity of stool, urine and ascitic fluid specimens was assayed in 100 ml. samples in Marinelli beakers placed around bismuth walled or tetramethyl lead filled silver walled glass Geiger counters with sensitivities of 5200 counts per minute per μc I^{131} above a background of 28 counts per minute and of 479 counts per minute per μc I^{131} above a background of 16 counts per minute, respectively. Plasma and ascitic fluid were assayed in 5 ml. samples in specially modified Marinelli-type beakers placed around the bismuth tube. A sensitivity of 10,500 counts per minute per μc I^{131} was obtained with this arrangement. Duplicate 0.5 ml. plasma samples were dried in circular planchets of 2 cm. diameter and assayed in a flow gas counter. The sensitivity of the arrangement was 1.02 to 1.10×10^6 counts per minute per μc I^{131} depending on the density of the plasma, above a background of 20 counts per minute. The sensitivity could be precisely determined for each subject's plasma by the addition of a known amount of I^{131}. Five ml. samples were also occasionally assayed in a well-type sodium iodide crystal scintillation counter with a sensitivity of 0.96×10^6 counts per minute per μc I^{131} for this volume, above a background of 432 counts per minute. Suitable dilutions of the administered doses were assayed in all counters and kept throughout the study for frequent intercomparisons and for verification of stability of the various counting arrangements. In a number of experiments all plasma samples were assayed in both the flow gas and bismuth counters and some samples assayed in the scintillation counter as well. The precision obtained in replicate samples assayed in different counters indicated that counting techniques were not a source of significant variation. A sufficient number of counts was recorded so as to reduce the statistical error of counting to below 3 per cent in the least active plasma specimens.

Subjects studied. These were male patients of the Veterans Administration Hospital, Bronx, New York. One hundred to 150 μc I^{131} tagged albumin (1 to 20 mgm.) from different lots were administered intravenously from weighed syringes to groups composed of one to seven subjects. The larger groups included cirrhotic and non-cirrhotic patients. There were 53 separate studies in 46 subjects. Inhibition of thyroid uptake was effected by the administration of 10 drops of Lugol's solution by mouth three times a day throughout the study. 24 hour urine collections were obtained daily except in an occasional instance when a pooled weekend urine was collected. Heparinized blood specimens were drawn without tourniquet stasis at frequent intervals for periods up to 43 days following administration of the labeled albumin. While in many early experiments blood samples were drawn from the recumbent subject after rest, this practice was subsequently discontinued as an unnecessary refinement in long term studies. Serum albumin concentrations were determined at 7 to 10 day intervals from micro Kjeldahl nitrogen analysis after Howe sodium sulfate fractionation. Ascitic fluid albumin concentrations were similarly determined. In all but one case (J. B., No. 10), these concentrations remained constant within the limits of error of the method. Plasma volumes were determined from the 15 minute dilution factors according to methods previously described (10).

Localization of radioactivity in plasma and ascitic fluid. The estimation of turnover rates from the analysis of plasma radioactivity requires that the radioactivity remain bound to the undegraded albumin. This condition has been examined by the dialysis of plasma, the precipitation of plasma proteins and the assay of radioactivity in electrophoretically fractionated plasma at various intervals following the administration of tagged albumin. In all experiments less than 4 per cent of the plasma radioactivity passed a dialysis membrane, more than 94 per cent of the radioactivity was precipitable with cold 10 per cent trichloracetic acid and all of the radioactivity was accounted for in the albumin fraction separated by electrophoresis. It should be appreciated, however, that separation by this latter method is crude and that this fraction undoubtedly also contained some alpha-globulin. Approximately 98 per cent of ascitic fluid radioactivity was precipitable with cold 10 per cent trichloracetic acid.

Definition of terms.

Rate—Apparent instantaneous fractional rate; this may be an average of several indistinguishable rates.

Compartment—An apparent space of distribution not necessarily confined to a single anatomically bounded region except in the case of plasma.

Iodo-Albumin Component—A moiety of iodo-albumin composed of a single biologically homogeneous substance or of several different substances characterized by closely similar degradation rates.

Iodo-Albumin Degradation—The separation of radioactivity from the I^{131} labeled albumin which does not necessarily imply total or partial hydrolysis of the protein.

ANALYSIS OF DATA AND RESULTS

I. *Construction and analysis of curves*

The method of plotting observed and derived data and the significance of the curves obtained are best followed from the study of a representative experiment (Figure 1). In each case, the following curves were drawn. All values were corrected for radioactive decay and plotted as a function of time on semilogarithmic paper.

A) *"Plasma concentration curve."* Counts per minute per unit volume plasma. After distribution in the body's exchangeable albumin pool is essentially complete and in the absence of proteinuria or other external losses, the slope of this curve defines the rate of tagged albumin degradation. It has been inferred (3) that the rate of albumin synthesis is equal to this rate if the concentration and space of distribution of serum albumin remain constant. In one case (J. B., No. 10), be-

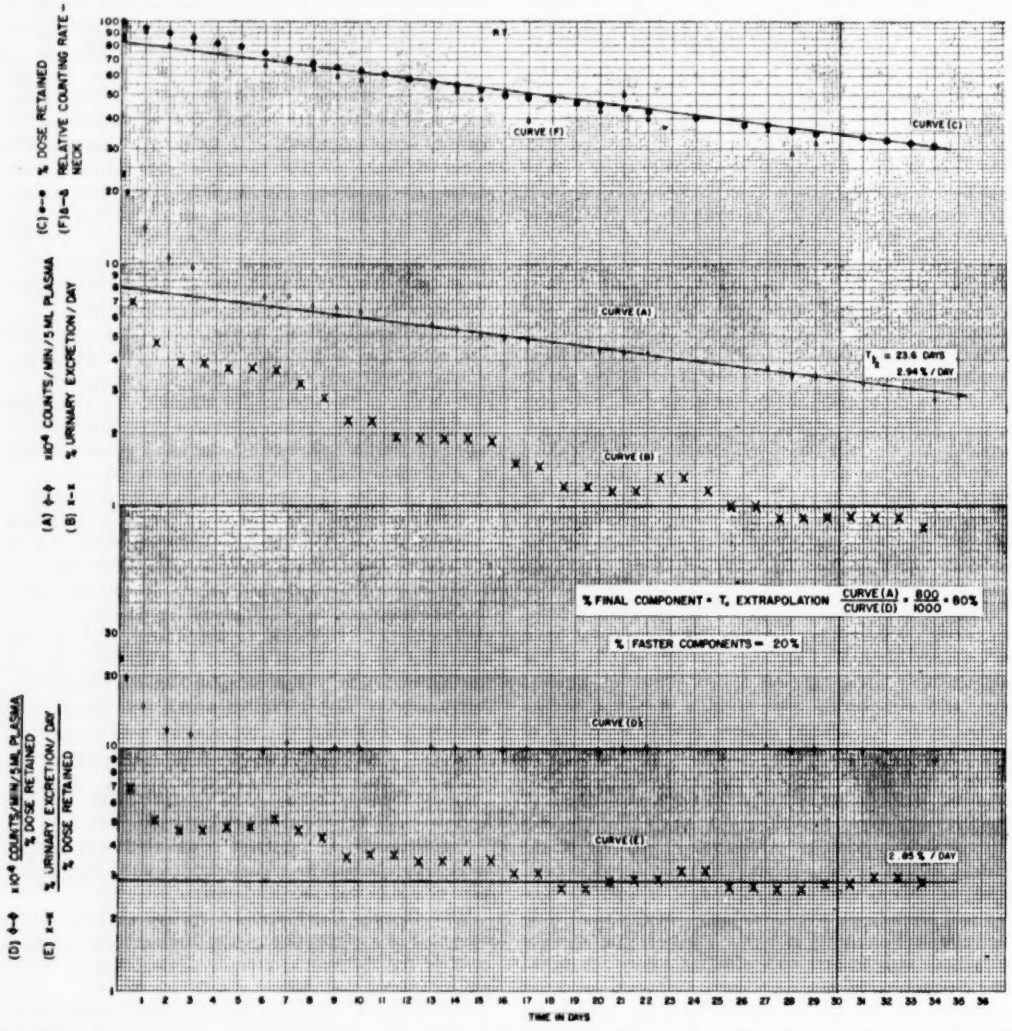

(C) ●—● % DOSE RETAINED
(F) ▲—▲ RELATIVE COUNTING RATE - NECK
(A) ◊—◊ ×10⁴ COUNTS/MIN/5 ML PLASMA
(B) ×—× % URINARY EXCRETION/DAY
(D) ◊—◊ ×10⁴ COUNTS/MIN/5ML PLASMA / % DOSE RETAINED
(E) ×—× % URINARY EXCRETION/DAY / % DOSE RETAINED
CURVE (F)
CURVE (C)
CURVE (A)
CURVE (B)
CURVE (D)
CURVE (E)
$T_{\frac{1}{2}}$ = 23.6 DAYS
2.94 % / DAY
% FINAL COMPONENT = $T_{\frac{1}{2}}$ EXTRAPOLATION CURVE (A) / CURVE (D) = 800/1000 = 80%
% FASTER COMPONENTS = 20%
2.65 % / DAY
TIME IN DAYS

FIG. 1. TYPICAL SET OF CURVES DERIVED FROM DATA IN SUBJECT R. T. (NO. 15) (SEE TEXT FOR INTERPRETATION)

In this and subsequent figures some of the urine points represent the means of two day collections. The first point represents the actual excretion divided by the fraction of a 24 hour period over which it was collected.

cause of a wide variation in plasma albumin concentrations (± 15 per cent of mean), specific activities (counts per minute per gram albumin) rather than radioactivities were plotted.

B) *"Renal excretion curve."* The per cent of the administered dose excreted daily in the urine is plotted at the midpoint of the day. In one case (S. H., No. 43) significant proteinuria was present and separate assay of the protein bound and protein free radioactivity was made.

C) *"Per cent retained."* The per cent of the dose retained in the body at any time is considered to be equal to 100 per cent minus the cumulative per cent urine excretion on the assumption that all the radioactivity lost from the body is recovered in the urine collections. The validity of this assumption is confirmed or negated, in any individual case, by the characteristics of the forms of curves D) and E) described below. Approximately 2 to 3 per cent of the radioactivity was withdrawn during blood sampling during the course of the study. These losses were corrected for in calculation of the per cent retained. In three subjects occasional 24 hour stool analyses showed that fecal excretion amounted to only .2 per cent to 2.5 per cent of that excreted in the urine. Fecal excretion was therefore disregarded.

D) *"Distribution curve."* Counts per minute per unit volume plasma/per cent retained. On the assumption that all radioactivity released from the degraded protein is quantitatively accounted for by renal excretion, this curve reflects the changes in plasma concentration due solely to distribution of the tagged albumin in the exchangeable albumin pool. If the assumption is valid, this curve should approach an asymptote parallel to the time axis as mixing becomes more complete. It should have a negative slope if recovery in the urine does not account for all the degraded protein radioactivity. The reciprocal of this curve would indicate the time course of the apparent space of distribution.

E) *Daily renal excretion (per cent of dose)/per cent retained.* If the assumption made in C) is valid, then the absolute level of this curve at any time expresses the per cent of the tagged protein within the body which is being degraded daily at that time.

F) *In vivo counting rate over neck and/or other parts of the body.* After distribution is essentially complete, these curves should parallel the plasma concentration curve, provided that selective localization is not taking place in the site under observation. Assay over the neck also served to confirm the inhibitory effect of Lugol's solution on thyroid uptake of I^{131}.

Renal excretion of radioactivity released from the degraded protein

A consideration which merits attention is the time relationship between the urinary excretion and plasma concentration curves. The extent to which the lag in the urinary excretion of the degraded protein radioactivity affects the curves was evaluated. It can be shown that the rate of appearance of radioactive iodine in the urine rapidly approaches its rate of release from the degraded labeled albumin (Appendix A). Thus, urinary excretion may be accepted as simultaneous with iodo-albumin degradation for the purpose of analysis.

Significance of the "distribution curve"

Analysis of the observed plasma concentration curves reveals a progressively decreasing slope for 4 to 7 days or longer depending on the batch of albumin used. Eventually a straight line could reasonably be drawn through the terminal segments of the curve. Inspection of the distribution curve (Figure 1D) reveals that an horizontal asymptote is reached at the 5th or 6th day. Thus, the decline in the plasma concentration curve after this time is quantitatively accounted for by urinary excretion, indicating that the volume of distribution is no longer increasing. The significance of the horizontal asymptote in the distribution curve is twofold:

1. It offers evidence that during the entire period of study, radioiodine released from degraded protein is almost completely recovered in the urine. If any radioactivity were not recovered, the calculated value for the per cent retained would be higher than the true one by that amount not recovered. The percentage error would then progressively increase during the course of the study producing a negative slope in the curve.

2. It further indicates that any change in the slope of the plasma concentration curve after the distribution curve has become horizontal must be due to a change in the apparent rate of degrada-

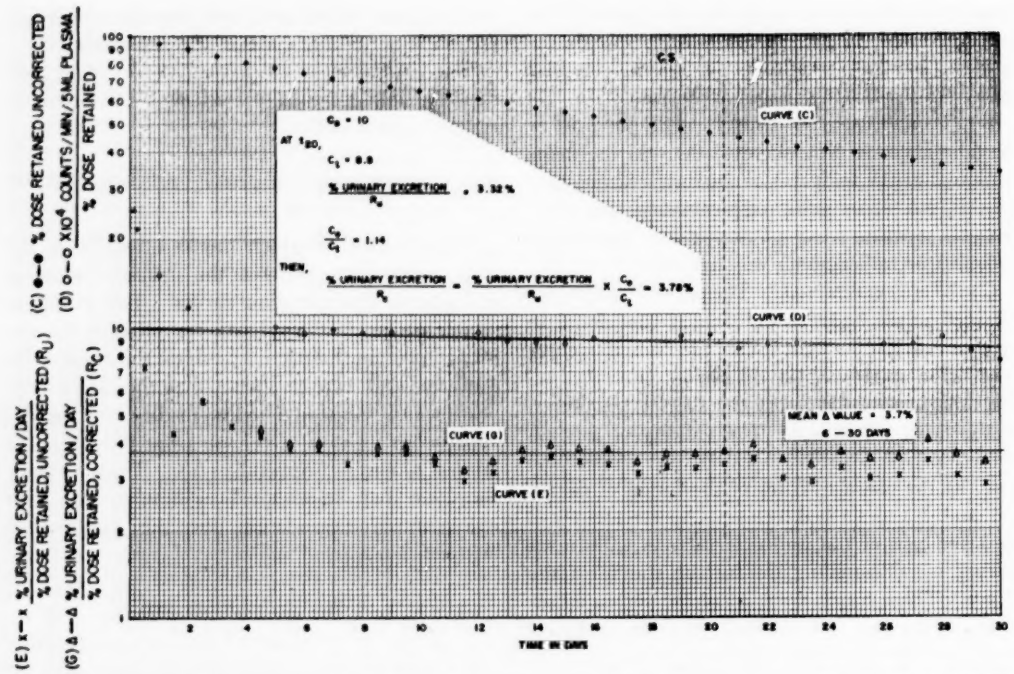

(C) ●—● % DOSE RETAINED UNCORRECTED
(D) ○—○ X10⁴ COUNTS/MIN/5ML PLASMA / % DOSE RETAINED
(E) x—x % URINARY EXCRETION/DAY / % DOSE RETAINED, UNCORRECTED (R_U)
(G) Δ—Δ % URINARY EXCRETION/DAY / % DOSE RETAINED, CORRECTED (R_C)
C.S.
CURVE (C)
CURVE (D)
CURVE (G)
CURVE (E)
C₀ = 10
AT t₈₀,
C_t = 8.8
% URINARY EXCRETION / R_U = 3.38%
C₀/C_t = 1.14
THEN,
% URINARY EXCRETION / R_C = % URINARY EXCRETION / R_U × C₀/C_t = 3.78%
MEAN Δ VALUE = 3.7%
6 — 30 DAYS
TIME IN DAYS

FIG. 2. METHOD OF CORRECTION FOR LOSSES NOT ACCOUNTED FOR IN URINE COLLECTIONS IN SUBJECT C. S. (No. 7)

The calculation for the correction of $\frac{\text{Renal Excretion}}{\text{Per cent Retained}}$ for the 21st day is shown. The mean for the corrected values = 3.7 per cent.

tion and is not to be explained by distribution or storage phenomena.

The observation that urinary excretion accounts for all the degraded protein radioactivity allows for the calculation of the rate of degradation from the urine assays alone. The expression

$$\frac{\text{renal excretion per unit time}}{\text{per cent retained}}$$

defines this rate at any time. Degradation rates determined from two independent sets of data, plasma concentrations and urinary excretions, may thus be compared.

In many cases the distribution curve had a slight negative slope even after apparent distribution equilibrium (Figure 2). Theoretically, this could be explained by continued distribution into less easily penetrated compartments of an albumin pool, or by failure of recovery of all degraded protein radioactivity in the urine, owing either to incomplete urine collection or to extrarenal losses. Continued distribution over the entire period is almost certainly not the explanation, since in that event an approach to a horizontal asymptote rather than a progressive decline would have been observed. In several cases the observations were continued for a period long enough to recover over 85 per cent of the administered doses in the urine when the plasma still contained an additional 5 to 7 per cent. Furthermore, since fecal excretion has already been noted to be insignificant and, in many cases, urinary recovery did account for all losses from the body, it is probable that incompleteness of the urine collections is responsible for the slope observed in these curves. Where such slopes were observed, appropriate correction for the amount not recovered was made as follows (see Figure 2):

Let

C_0 = zero time extrapolation of the final slope of distribution curve,

C_t = point on this slope at time t,

R_u = per cent retained, uncorrected, time t = 100% − (cumulative per cent urinary excretion + per cent withdrawn by blood sampling),

R_c = per cent retained, corrected, time t.

Then

$$R_c = \frac{C_t}{C_0} \times R_u \qquad (1)$$

The renal excretion values may be corrected as follows:

$$\frac{\%\text{ renal excretion}}{R_c} = \frac{\%\text{ renal excretion}}{R_u \times \frac{R_c}{R_u}} \qquad (2)$$

From (1) and (2)

$$\frac{\%\text{ renal excretion}}{R_c} = \frac{\%\text{ renal excretion}}{R_u} \times \frac{C_0}{C_t}$$

II. *Kinetics of distribution into albumin pool*

The distribution curve, rather than the observed plasma concentration curve is used for analysis of the kinetics of distribution in order to exclude the effects of variation in the rates of iodo-albumin degradation during this period. Even here this analysis remains in error to the extent that urinary excretion lags somewhat behind protein degradation (Appendix A). While this could have been more fully accounted for by a separate study of the renal iodide clearance rate, the error involved was not great enough to justify the complicated mathematical treatment required of this refined analysis.

For the purpose of determining more precisely the rates of distribution, 10 to 16 blood samples were drawn during the first 3 to 4 days in about one-fourth of the cases (Figure 3). However, these rates could be grossly evaluated in the remaining cases as well.

Although there may be an unlimited number of exponential components, the distribution curves, in most instances, could be resolved into three major exponential regression rates by conventional analysis (Figure 3). If mechanical mixing in the plasma is considered to be complete at t_0, the form of these curves is then expressed by the gen-

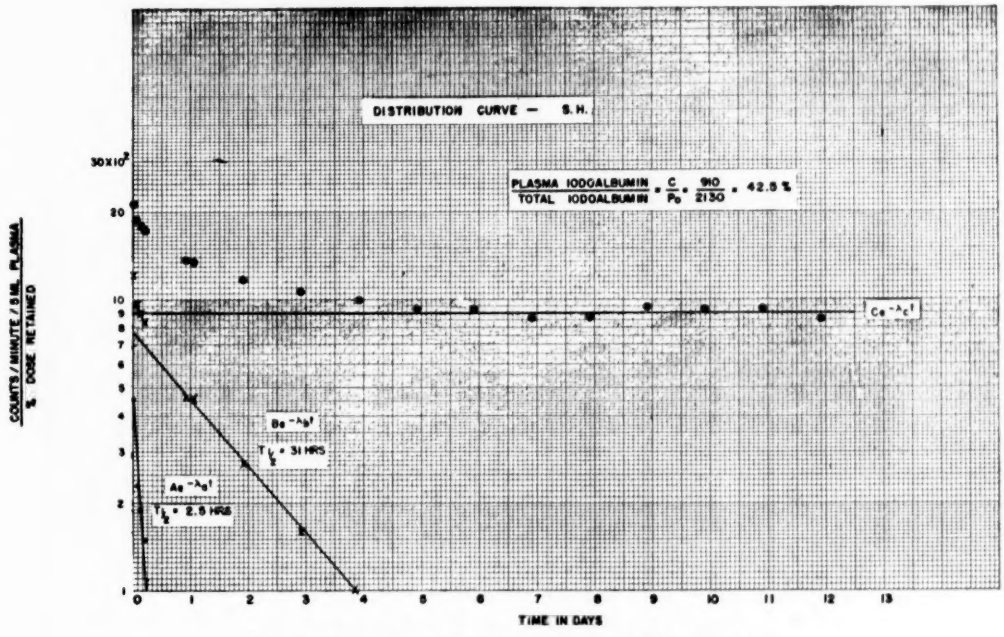

DISTRIBUTION CURVE — S. H.
PLASMA IODOALBUMIN / TOTAL IODOALBUMIN = C/P0 = 910/2130 = 42.8 %
Ce −λc t
Be −λb t
Tb = 31 HRS
Ae −λa t
Ta = 2.5 HRS
COUNTS / MINUTE / 5 ML. PLASMA / % DOSE RETAINED
TIME IN DAYS

FIG. 3. DISTRIBUTION CURVE IN SUBJECT S. H. (No. 43)

This figure demonstrates the method of analysis for the rates of equilibration between plasma and the "fast" and "slow" extravascular compartments and for the fraction of total iodo-albumin in circulating plasma at equilibrium.

TABLE I

DATA PERTAINING TO DISTRIBUTION AND DEGRADATION OF I^{131} LABELED ALBUMIN

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S			T
											DATA FROM PLASMA CONCENTRATION CURVE							% / Day Urine excretions—first three days			
Method of preparation and lot number	Exp. No.	Subject	Clinical Diagnosis	Age	Weight kg	Mean serum albumin conc. gms/100 ml	Plasma volume ml	% Iodoalbumin extravascular	TEA	TEA / kg	Days to apparent constant slope	Period of observation	$T_{\frac{1}{2}}$	% / day	Albumin degradation gm/day	gm/day/kg	Degradation rate from renal excretion % /day	1	2	3	Fraction faster components
A																					
LOTS OF ALBUMIN FREE FROM EXCESSIVE AMOUNTS OF RAPIDLY DEGRADED COMPONENTS																					
B 1-26-3 III A/M	1	J.D.	Parox. Aur. Fib.	53	94	3.84	3280	63	350	3.7	9	32	11	6.3	22.1	.234	6.3	7.3	6.9	6.9	$\frac{1}{2}$
16I A/M	2	M.F.	Diverticulosis	57	52	3.74	2100	60	197	3.8	4	32	16	4.3	8.5	.164	4.0	3.3	2.8	3.4	$< \frac{1}{20}$
	3†	F.A.	C.D.A.	44	64	2.39	3800	61	233	3.6	5	16	>24	<2.9	<6.7	<.105	<0.5	1.0	1.7	1.8	
	4†	G.H.	C.D.A.	54	84	2.53	4560	70	385	4.6	9	42	>24	<2.9	<11.1	<.133	1.8	4.1	3.1	2.8	
B 1-7-3	5	J.W.	Rheumatoid arthritis	31	74	3.86	3730	59	351	4.8	6	42	18	3.9	13.7	.185	3.7	5.2	4.9	4.4	0
	6	H.P.	C	64	89	3.38	4100	58	330	3.7	5	42	18	3.9	12.9	.145	3.7	3.8	1.9	3.2	
	7	C.S.	Rheumatoid spondylitis	35	61	4.12	3030	60	312	5.1	5	30	18	3.9	12.2	.198	3.7	6.0	4.0	5.0	
	8	M.B.	Conv. duodenal ulcer	50	57	3.63	2985	59	264	4.6	5	29	17.5	3.96	10.4	.171	4.0	6.0	5.4	4.7	
	9	S.M.	C.D	48	70	2.96	4740	48	270	3.9	16	35	19	3.6	9.7	.139	3.2	3.5	4.4	3.6	
	10	J.B.	Diabetes M.	64	70	3.75 (3.31–4.33)	2850	74	411	5.9	6	35	25	2.8	11.5	.165	2.7	3.5	3.9	4.2	
	11	P.McG	Senile emphysema	67	46	3.97	2880	67	347	7.5	14	34	19	3.6	12.6	.275	3.0	1.9	2.9	4.6	
B 12-2-2	12	J.G.	Buerger's disease	34	66	4.45	2530	55	250	3.8	7	42	15	4.6	11.5	.175	4.6	4.9	6.3	5.3	$0-\frac{1}{5}$
	13†	O.C.	C.D.A.	58	71	2.55	4210	62	283	4.0	3	17	>21	<3.3	<9.4	<.132	<1	1.2	1.7	1.8	
	14†	H.D.	C.D.A.	40	80	2.76	3200	73	327	4.1	14	35	36	1.9	6.3	.079	1.9	2.3	3.4	3.4	
	15	R.T.	C.D.	42	66	2.32	3550	55	183	2.8	12	35	23.6	2.94	5.4	.082	2.85	5.8	4.7	4.3	
A 5-18-2	16	E.C.	Normal	34	74	4.80	2825	65	388	5.3	5	23	18	3.9	15.1	.204	D.I.	4.3	3.5	2.3	$< \frac{1}{10}$
	17	H. Wtd	C.	40	96	4.20	4240	54	386	4.0	10	28	14.5	4.8	18.5	.192	5.1	6.4	5.4	4.6	
A 5-1-2	18	G.S.	Cardiospasm	39	64	4.44	3170	58	335	5.2	4	23	18	3.9	13.1	.204	2.5	4.8	4.4	4.0	$< \frac{1}{10}$
	19	J.O.	Conv. duodenal ulcer	22	68	5.07	3600	54	397	5.8	4	17	13	5.3	21.1	.310	3.5	4.8	5.6	3.5	

B

LOTS OF ALBUMIN CONTAINING APPRECIABLE AMOUNTS OF RAPIDLY DEGRADED COMPONENTS

		20	E.Y.	Senile osteoporosis	69	66	3.89	3050	67	360	35	10	30	15	4.6	16.6	.252	D.I.	11.0	7.3	7.6	
		21	S.C.	Conv. duodenal ulcer	38	75	4.44	3600	66	470	6.3	14	25	18	3.9	18.1	.260	3.5	7.7	7.6	5.0	
A	B-28-2	22	M.A.	Post phlebitic syndrome	42	97	4.07	3220	65	375	3.9	8	26	15	4.6	17.2	.178	5.0	9.0	9.5	5.5	1/3
		23	R.G.	Psychoneurosis	32	93	4.50	3710	53	355	3.8	5	14	10	6.9	24.6	.264	5.0	12.3	9.3	6.5	
		24	Ge.H	C. D.	35	89	3.75	4850	54	395	4.4	13	43	18	3.9	15.2	.171	4.7	13.3	9.6	6.0	
		25	F.B.	Rheum. heart dis. compensated	35	77	4.61	3040	63	379	4.9	11	22	12	5.8	21.8	.284	5.0	13.4	8.9	4.9	
ABBOTT	A254-13	26	A.J.	Rheumatoid arthritis	58	58	3.70	3700	69	440	7.6	6	22	9.0	7.7	34.8	.583	5.9	12.1	10.3	6.5	1/3
		27	E.M.	C.	45	64	3.55	3410	59	295	4.6	11	22	11	6.3	18.6	.290	5.1	9.4	8.8	6.9	
		28	E.B.	Conv. Scurvy	39	61	4.17	3400	60	354	5.8	15	23	19	3.6	12.9	.212	4.0	9.5	8.5	3.9	
	A 5-22-2	29	F.K.	C.*	38	87	5.07	3800	65	550	6.3	9	16	10	6.9	38.0	.440	4.5	6.8	5.5	6.8	1/3
		30	C.R.	Thrombophlebitis	29	74	4.53	3000	59	331	4.5	7	23	11	6.3	20.7	.282	4.5	10.8	9.4	5.4	
ABBOTT	A223-2	31	G.A.	P. vera	40	68	4.57	3820	68	546	8.0	5	15	13	5.3	29.2	.430	D.I.	22.4	D.I.	D.I.	1/3
		32	C.D.	P. vera	31	80	3.85	3220	62	326	4.1	14	21	13	5.3	17.8	.222	5.1	15.4	11.2	8.7	
		33	J.McD.	Diabetes M.	34	82	4.69	2850	71	460	5.6	14	21	15	4.6	21.2	.258	3.3	15.6	8.2	3.0	
ABBOTT	A216-2	34	F.M.	Conv. duod. ulcer	40	82	4.62	3470	60	400	4.9	17	16	12	5.8	23.1	.282	3.8	21	9.8	5.6	1/3 — 1/2
		35	O.B.	Diabetes M.	42	75	4.38	3940	60	430	5.7	7	21	14	4.9	21.2	.283	4.2	17.7	11.7	5.2	
		36	G.P.	C.	38	85	4.41	3740	58	393	4.6	10	21	13	5.3	20.8	.245	3.6	21	11.3	3.8	
		37	G.O.	C.D.	52	75	3.32	5800	—	—		14	21	24	2.9	—	—	D.I.	12.6	D.I.	D.I.	
ABBOTT	A150-4	38	A.S.	Conv. duod. ulcer	42	90	4.50	3670	53	351	3.9	7	14	14	4.9	17.3	.193	5.7	18	11.2	8.4	1/2
ABBOTT	A143-2	39	W.S.	Pernicious Anemia	52	68	4.37	2350	70	342	5.0	16	24	14	4.9	16.7	.246	4.8	11	11	6.8	1/2
ABBOTT	A137-5	40	J.H.	A.S. Heart Dis. no edema	62	75	4.06	3130	70	424	5.7	9	23	13.5	5.1	21.6	.288	3.6	9.3	7.3	5.0	1/3
		41	A.B.	C.D.	37	70	3.65	4750	52	362	5.2	13	22	16	4.3	15.6	.222	3.3	10.5	8.5	4.9	
ABBOTT	A131-3	42	H.H.	C.	34	68	3.51	3660	60	326	4.8	7	21	14	4.9	16.1	.237	4.0	9.2	7.5	6.0	1/3
ABBOTT	A111-2	43	S.H.	Ch. glom. nephr.	31	60	4.18	2700	58	268	4.5	9	25	14	3.7Δ	9.9Δ	.165Δ	4.1Δ	8.9	9.0	8.6	1/2
ABBOTT	A445-196	44	R.J. (a)	Rheumatoid Arth.	43	85	4.00	3900	61	400	4.7	8	18	13.5	5.1	20.4	.240	4.3	5.8	6.5	4.5	1/2
ABBOTT	A065-11	45⊖	(b)	" " "	43	85	4.10	3140	62	338	4.0	3	18	9	7.7	26.0	.306	6.1	11.2	9.8	7.6	1/2
ABBOTT	A117-2	46⊖	(c)	" " "	43	78	3.76	3400	61	327	4.2	5	17	9.5	7.3	23.9	.306	7.3	8.2	11.6	7.3	1/2
ABBOTT	A445-142	47	O.S.(a)	" " "	36	70	4.27	3230	56	314	4.5	7	13	17	4.1	12.8	.183	5.0	11	7.1	5.5	1/3
		48	F.Z.(a)	" " "	52	62	3.62	3620	54	285	4.8	5	13	15.5	4.5	12.7	.205	3.8	7.3	9.6	6.0	
ABBOTT	A445-178	49⊖	O.S.(b)	" " "	36	70	4.26	3130	55	295	4.2	4	16	13	5.3	15.7	.224	5.5	8.6	7.3	6.5	1/10
		50⊖	F.Z.(b)	" " "	52	62	3.62	3690	51	272	4.4	5	10	11	6.3	17.1	.276	5.6	8.6	7.4	6.1	
ABBOTT	A445-128	51	H.W.(a)	C.	40	98	3.50	4360	50	305	3.1	6	15	13.5	5.1	15.6	.160	7.8	12	6.7	11	1/3
ABBOTT	A445-147	52	(b)	C.	40	96	3.50	4580	46	298	3.1	10	19	14	4.9	14.7	.153	4.0	6.3	6.2	6.0	1/3
ABBOTT	A014-2	53	(c)	C.	40	96	3.70	4450	53	351	3.7	6	30	11	6.3	22.1	.230	5.5	4.8	9.4	5.5	1/10

C—Hepatic cirrhosis, compensated.
CD—Hepatic cirrhosis, decompensated without detectable ascites.
CDA—Hepatic cirrhosis, decompensated with ascites.
conv—Convalescing.
D.I.—Data insufficient.
†—Ascites increasing during observation period.
‡—Ascites not increasing during observation period.
*—In spite of repeated high serum albumin concentration, diagnosis proven by biopsy.
Δ—25 per cent of urine radioactivity consistently in protein precipitable fraction—degradation rates calculated from non-protein bound radioactivity excretion.
⊖—Under Cortisone therapy during these studies. Because of uncertain reliability of these lots, the apparent increase in rate of degradation, as compared with control studies in the same subjects, while suggestive, is not considered established.

eral formula:

$$P = Ae^{-\lambda_a t} + Be^{-\lambda_b t} + Ce^{-\lambda_c t}$$

where

$P = \frac{\text{Plasma concentration}}{\text{Per cent retained}}$

λ_c[5] = Rate constant due to incomplete urinary recovery of degraded protein radioactivity. In many cases this equalled zero, as noted above.

λ_a and λ_b = Rate constants determined by transfer of tagged albumin between plasma and two apparent extravascular compartments.

λ_c is calculated from the slope of the terminal straight line phase of the curve. Subtraction of the values of this extrapolated line from the corresponding values of the curve produces a new curve. λ_b and λ_a are then determined in a similar manner from successive resolution of the curves so produced.

A, B and C = Coefficients obtained from the vertical axis intercepts of the regression lines, $Ae^{-\lambda_a t}$, $Be^{-\lambda_b t}$, $Ce^{-\lambda_c t}$, respectively.

At $t = t_0$, $P = P_0$. Then,

$P_0 = A + B + C$,

$\frac{C}{P_0}$ = fraction of total iodo-albumin in plasma at distribution equilibrium,

$\frac{A + B}{P_0}$ = fraction of total iodo-albumin in spaces other than plasma at distribution equilibrium.

Total plasma albumin = albumin concentration × plasma volume.

If iodo-albumin is assumed to exchange with endogenous albumin, then,

Total exchangeable albumin (TEA)

$$= \text{total plasma albumin} \times \frac{P_0}{C}.$$

Values for $\frac{A + B}{P_0}$ are given in column I of Table I. The mean value for compensated cirrhotic and non-cirrhotic subjects was 59.5 per cent. The somewhat higher percentage (mean 66.5 per cent) in the presence of ascites was to be expected. In case H. D. (No. 14), paracentesis on the last day of observations revealed that approximately 60 per cent of the total iodo-albumin was present in the 19 liters of ascitic fluid removed, and in case Gl. H. (No. 4) 42 per cent was present in 15.5 liters. In case F. A., (No. 3) 6.5 liters of ascitic fluid contained 16 per cent of the iodo-albumin in the body.

Values for TEA are given in column J of Table I. In many of the cirrhotic subjects TEA was not significantly lower than in non-cirrhotics. In these cases an increase in plasma volume or the presence of fluid accumulations compensated for the low serum albumin concentrations.

Extravascular compartments

In all but a few of the studies, two greatly different rates of distribution were observed with mean half times of approximately 3 and 24 hours. For lack of more precise information as to their localization, these two extravascular compartments are merely termed "fast" and "slow." In the possibility that these compartments were not diffusely distributed throughout the body, *in vivo* assays were performed in an attempt to localize sites of build-up of radio-activity.[6] However, no such sites were discovered by assays over the heart, lungs, liver, spleen, neck or thigh taken 15 minutes after injection and at frequent intervals thereafter. Since in the liver and spleen areas, the counter "saw" a relatively large amount of blood compared to extravascular tissues, these curves approximately paralleled the heart and plasma curves during the distribution phase (Figures 4, 7). On the other hand, the neck and thigh curves followed more closely the curve of per cent dose retained (Figures 4, 1). Only over the mid-abdomen in some patients with ascites was there any suggestion of a "build-up" curve indicative

[5] Theoretically, λ_c is not a constant whether the loss is a single loss or distributed throughout the course of the experiment. However, in practice, the losses were of such slight degree that λ_c could be treated as a constant without significant error.

[6] It is postulated that these two compartments communicate directly with the plasma rather than end-wise with each other although it is to be appreciated that distinction between these two arrangements cannot be made from the plasma observations alone.

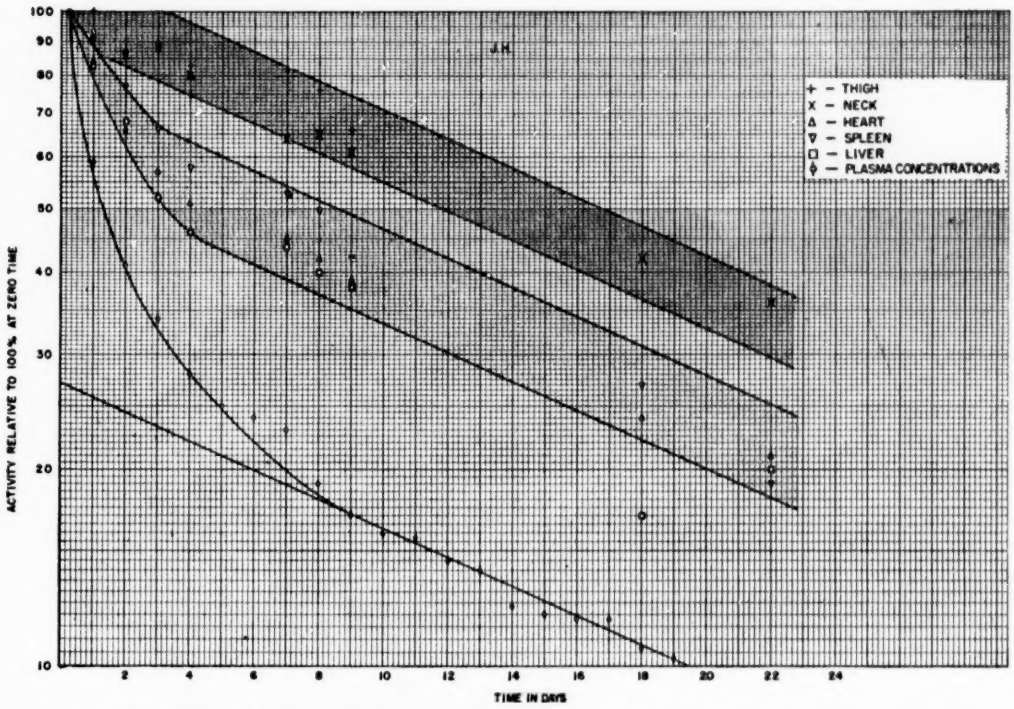


FIG. 4. SUBJECT J. H. (No. 40). TIME COURSE OF RADIOACTIVITY IN PLASMA AND IN VARIOUS BODY SITES
The stippled envelopes emphasize differences between areas which are exceptionally vascular and those which are not.

of localization. After distribution equilibrium was reached, the slopes of all these curves were approximately the same as that of the plasma concentration curves.

Removal of iodo-albumin from tissue sites

It is relevant to recall the studies of Cope and Moore (11) in dogs. By direct lymph analysis they demonstrated a much more rapid equilibrium between plasma and thoracic duct lymph than between plasma and lymph from the cervical and leg chains. Some evidence in human subjects is obtained from the rates of disappearance of tagged albumin from sites of injection into muscle, skin and subcutaneous tissue of the lower extremity. The half times for removal from these sites in 16 experiments ranged from 13 hours (Figure 5) to 72 hours (previously published figure [9]) which are consistent with the "slow" rate of exchange determined by the plasma studies.

From the observations presented here, no conclusions can be derived as to the presence of an intracellular phase except to note that *in vivo* assays failed to give evidence for localization in the liver as had previously been demonstrated for tagged modified human globin (9).

Iodo-albumin transfer between plasma and ascitic fluid

In one subject (Gl. H.), repeated sampling (20 to 30 ml.) of ascitic fluid was performed. During the period of this study, the ascitic fluid volume was increasing only slightly as judged by observation and daily weighings. Plasma and ascitic fluid distribution curves are shown in Figure 6. The ascitic fluid concentrations appeared to approximate a single exponential growth curve with a rate constant of 0.0150 per hour ($T_{1/2}$ = 46 hours). Theoretically, however, exchange between plasma and extravascular pools other than

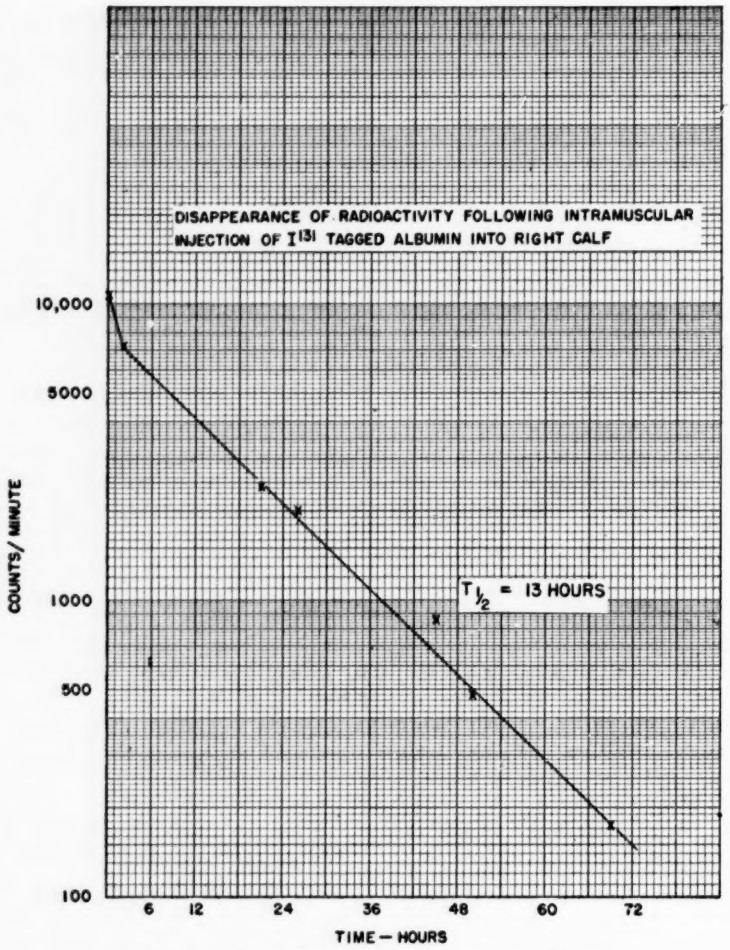


FIG. 5. DISAPPEARANCE OF IODO-ALBUMIN FROM MUSCLE

This was the shortest half-time observed. In most experiments the half-times for removal from skin, muscle or subcutaneous tissue of the lower extremity ranged from 18 to 36 hours.

the peritoneal cavity makes the situation much more complex. Because of this and the lack of appropriate observations of exchange rates and volumes of these compartments, the analysis of the kinetics of mixing was simplified by assuming a single rate of transfer into ascitic fluid. The dynamics of albumin exchange across the peritoneal membrane were evaluated in this manner under each of two extreme conditions (Appendix C). The calculations reveal that approximately 0.8 gram albumin passed the peritoneal membrane in each direction per hour.

III. *Degradation of tagged albumin*

As previously indicated, the slope of the plasma concentration curve following the distribution phase is a function of the rate of degradation of iodo-albumin. However, this portion of the curve was a straight line in only a small number of cases. These cases were limited almost exclusively

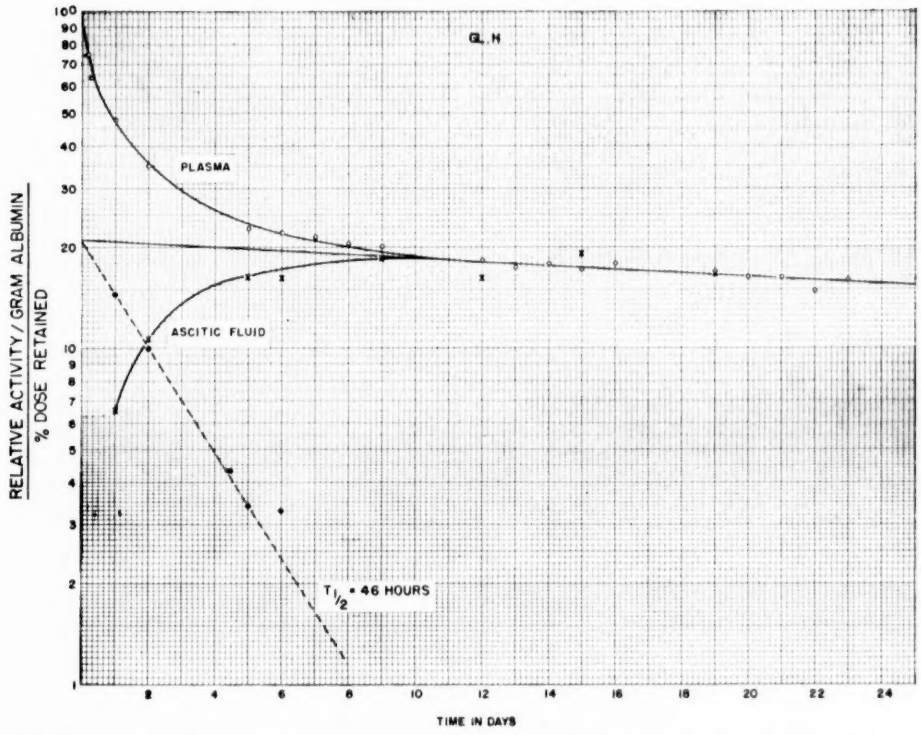


FIG. 6. CURVE OF EQUILIBRATION BETWEEN PLASMA AND ASCITIC FLUID IN SUBJECT GL. H. (NO. 4)

Equal specific activity in plasma and ascitic fluid was apparently reached in 10 days. The dotted line is a plot of the differences between the extrapolated equilibrium value and the observed ascitic fluid concentrations.

to lots prepared by method B. In these, the renal excretion curve, after the fifth or sixth day, was also a straight line and had a slope similar to that of the plasma concentration curve (Figure 7). The curve of $\frac{\text{renal excretion}}{\text{per cent retained}}$ remained at a horizontal level after distribution equilibrium was reached. The rates of degradation of the iodoalbumin as determined from the slopes of the plasma concentration curves were in close agreement with the values obtained from the horizontal portions of the $\frac{\text{renal excretion}}{\text{per cent retained}}$ curves (Table IA). In these cases, therefore, the observations are consistent with the interpretation that nearly all of the labeled albumin was being degraded at a single constant rate, which could be independently derived from plasma or urine analyses. During the phase of distribution, $\frac{\text{renal excretion}}{\text{per cent retained}}$ was slightly higher than during the subsequent flat portion of the curve.

Unfortunately, however, these conditions did not obtain in the majority of cases. The following characteristics of the post distribution segments of the curve were frequently observed (Figure 1). The slope of the plasma concentration curve revealed a gradual decrease. The slope of the renal excretion curve was greater initially but tended to approach that of the plasma concentration curve terminally. The curve of $\frac{\text{renal excretion}}{\text{per cent retained}}$ did not approach a horizontal level for some time after distribution was complete but rather showed higher levels in the initial portions of the post distribution segments. In these cases, therefore,

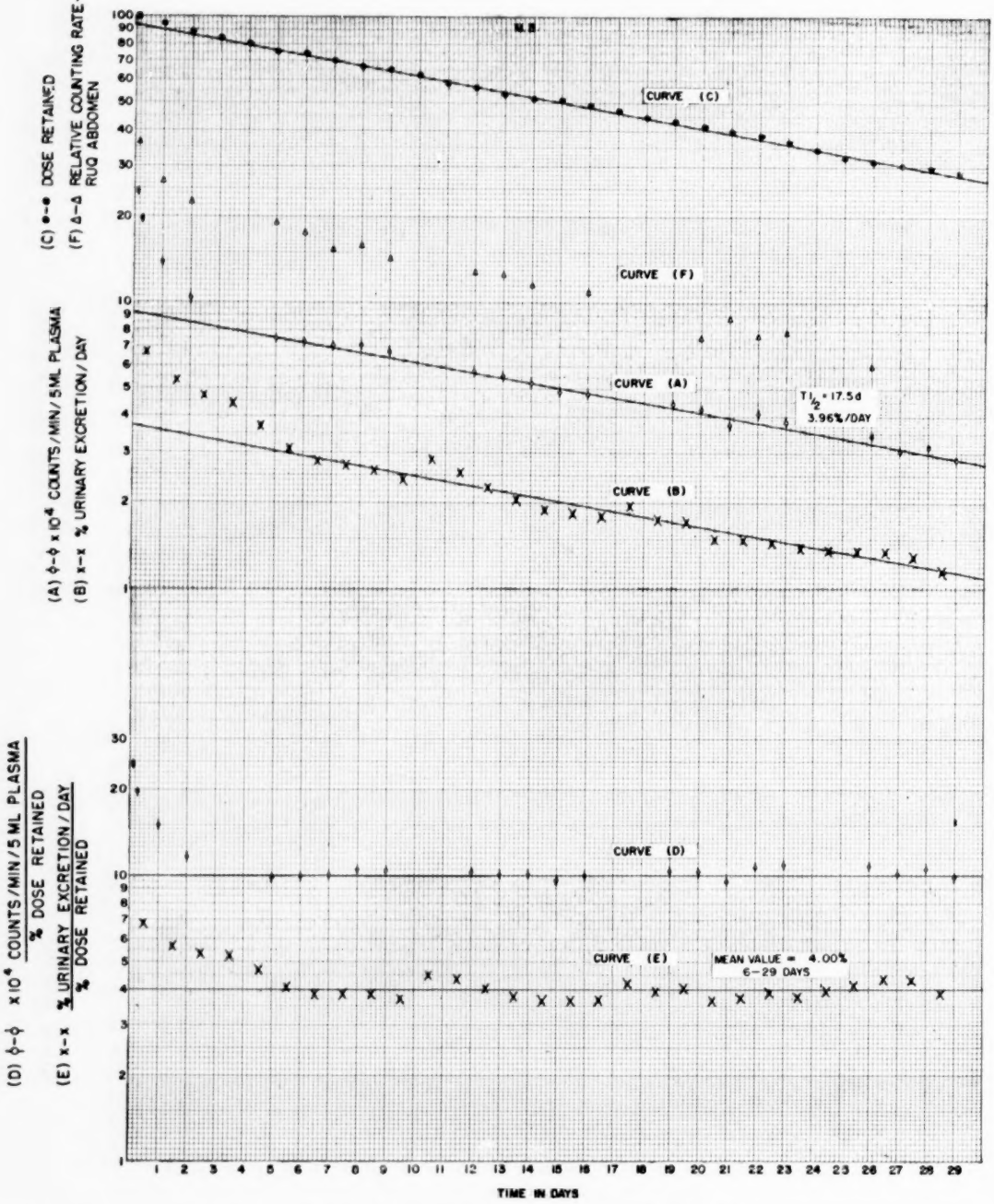


FIG. 7. SET OF CURVES DERIVED FROM DATA IN SUBJECT M. B. (No. 8)
In all cases in which this lot (B 1-7-3) of albumin was used, a single rate of degradation was observed.

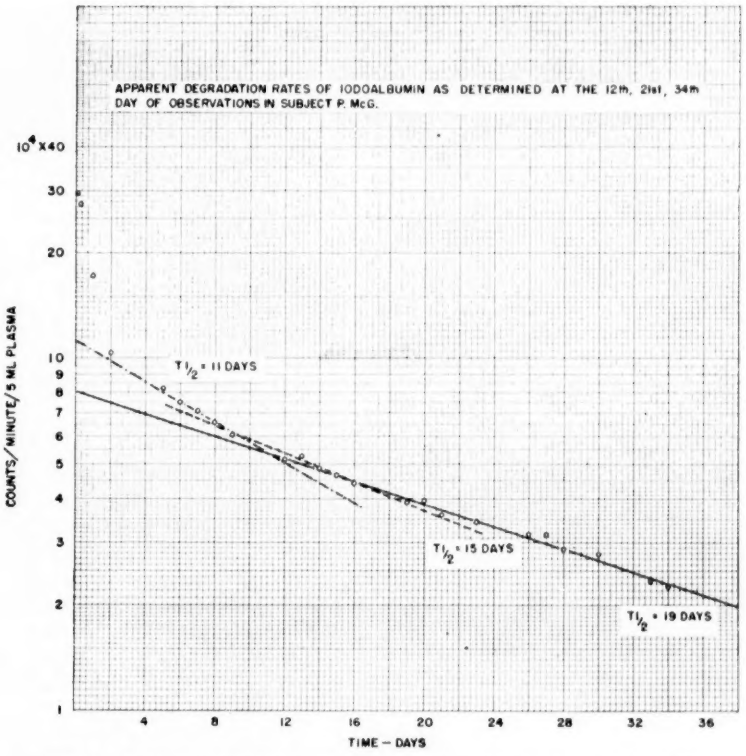
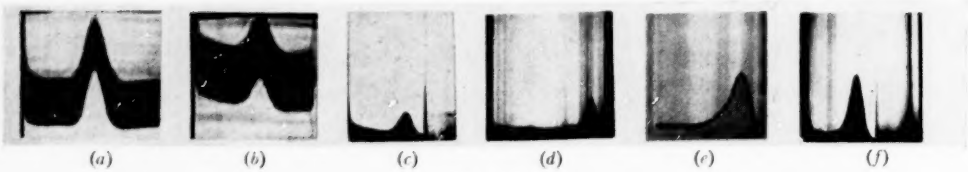


FIG. 8. PLASMA CONCENTRATION CURVE IN SUBJECT P. McG. (No. 11)
Had this experiment lasted only three weeks or less, a spuriously rapid degradation rate would have been obtained.

it is evident that degradation of the iodo-albumin was not proceeding at a single constant rate, but that there were several components being degraded at different rates. Theoretical curves for plasma concentration and renal excretion, when constructed for a two-component system, exhibit just such characteristics as have been observed in these studies (Appendix B).

As an example of the difficulties encountered in attempting to evaluate the rate of tagged protein degradation from most plasma concentration curves, the best straight line was drawn through the observed points in various phases of the study in subject P. McG. No. 11 (Figure 8). As the experiment proceeded the rate appeared progressively slower until the last half time of 19 days (3.6 per cent per day) was obtained for the 14th to 34th days after administration of I^{131} tagged albumin. In many cases it was two weeks or longer (Table I, column L) before the degradation rate became constant. Thus a minimum of three to four weeks was often required for evaluation of this final rate.

The fractional quantity of the last (slowest) component was estimated by dividing the zero time extrapolation of the final component of the plasma concentration curve by the zero time extrapolation of the terminal segment of the distribution curve (Figure 1). The constancy of the rate of degradation of the slower component during distribution may be seriously questioned. However, since this fraction of the iodo-albumin was degraded at a relatively slow rate, the error was small and the values obtained were useful as rough approximations. The observations did not permit the more refined approach utilized in the analysis of the distribution curves. It was found that the fraction of iodo-albumin which was



(*a*) (*b*) (*c*) (*d*) (*e*) (*f*)

FIG. 9.

Ultracentrifugal patterns of some lots used in this study: (*a*) and (*b*)*. Uniodinated albumin (*a*) compared with iodinated albumin (*b*) from lot Abbott A-143-2 used in case W. S. (No. 39). Although the latter material showed 50 per cent rapidly degraded components, there are no significant differences between the two patterns and the sedimentation constants are identical ($S_{20} = 4.3$ Svedberg units); (*c*) Albumin prepared by Method A (lot 5-22-2) used in cases (Nos. 28, 29, 30) in which about one-third of the material was rapidly degraded. $S_{20} = 4.6$ S.U. units. There are no abnormalities in the sedimentation pattern; (*d*) Abbott albumin B-192-37. Although there is only one sedimenting peak recorded, the sedimentation constant ($S_{20} = 5.7$ S.U.) is higher than that of "normal" albumin (ca. 4.7). This material gave high urine excretions over the first 24 hours (Table II); (*e*) Lot B 1-26-3 prepared with 11.1 I atoms per molecule protein and used in subject J. D. (No. 1). The "shoulder" on the "heavy" side indicates the presence of heavier polydisperse material in the preparation. In addition the sedimentation rate of the major component ($S_{20} = 6.2$ S.U.) is higher than normal, indicating appreciable alteration; (*f*) Lot B 1-26-53 prepared with 1.6 I atoms per molecule protein. This material which was used in subject M. F. (No. 2) was prepared identically with that in (*e*) except for the lower iodine: protein ratio. The pattern and sedimentation constant ($S_{20} = 5.0$ S.U.) are normal; used in subject M. F. (No. 2). Direction of sedimentation to the left in all frames.

* We are indebted to Dr. Sam Sorof, Vet. Adm. Hosp., Bklyn., N. Y., for performing these two analyses.

rapidly degraded was characteristic of the lot of iodo-albumin used (Table I, column T). In general, the lots prepared in the laboratory by Method B were the most satisfactory and those obtained from Abbott Laboratories the least so with respect to minimizing the portion of these rapidly degraded components. The presence of large amounts of these rapid components was evident in the high urine excretions of radioactivity during the first few days (Table IB, column S). From experience gained with the different lots it became clear that when more than 6 to 7 per cent had been excreted during the first 24 hours, it was likely that more than one component would subsequently be observed. However, even a lower excretion during the first day did not always exclude the presence of more than one component. It may again be emphasized that ultracentrifugal analysis often failed to detect such abnormalities in the various lots (Figure 9 b and c). However, a more recent lot of Abbott I^{131} tagged albumin has shown slight abnormalities in the ultracentrifuge (Figure 9 d). This and other recent lots have also shown extremely high renal excretions for the first 24 hours (Table II). These cases have not been included in the present study.

TABLE II

Renal excretions after administration of some recent lots of iodo-albumin supplied by Abbott Laboratories

Lot No.	Date shipped	Subject	Renal excretion % of dose administered	
			1st 24 hours	2nd 24 hours
B-42-6	7-24-52	J. S.	9.4—not dialyzed before administration	
B-71-25	8-21-52	F. M.	13.1	
B-156-2	11-26-52	S. F.	18.8	
B-193-37*	1-8-53	C. C.	13.3	
		E. K.	19.2	
B-210-40	1-30-53	L. I.	10.7	
		I. B.	6.2	
B-221-27	2-17-53	F. B.	22.7	
B-233-2†	2-20-53	H. M.	4.1	4.2
B-241-34	3-2-53	E. I.	17.4	

* High sedimentation constant on ultracentrifugal analysis (Figure 9d).

† Special experimental lot.

Degradation rate of final (slowest) component of iodo-albumin

Where observations were continued over a period sufficiently long for the faster components to appear to be almost completely degraded, the rate of degradation of the final component was almost always less than 5.5 per cent per day ($T_{1/2} = 12.5$ days). Subject J. D. (No. 1) who received albumin with an average of 11 Iodine atoms per molecule is an exception. This case is discussed in greater detail below. In the case of those experiments with lots containing a single detectable component or only a small fraction of faster components and where observations over a four week period or longer were available, the rates were even much slower, being of the order of 4 per cent a day or less ($T_{1/2} > 17$ days) (Table IA). In these cases, too, the degradation rates determined independently from the plasma concentration and $\frac{\text{renal excretion}}{\text{per cent retained}}$ curves were in excellent agreement. If the daily degradation of endogenous albumin is accepted as the product of total exchangeable albumin and the degradation rate ($.693/T_{1/2}$ days) of iodo-albumin, then in this group, the albumin "turnover" per day ranged from 5.4 to 21.1 grams (.079 to .310 gram per kilogram per day) (Table IA). The values for cirrhotic patients (range .079 to .192; mean .126 gram per kg. per day) were lower than those for non-cirrhotic subjects (range .164 to .310; mean .205 gram per kg. per day). Even if calculated on the basis of "dry weights," the calculated "turnover" values would reveal low figures for the cirrhotic subjects. In two of the cirrhotic patients who died in cholemia and hepatic decompensation (O. C., No. 13 and F. A., No. 3) the rates of degradation could not be determined precisely from the plasma concentration curves since these patients were continually accumulating ascitic fluid during the study. This led to a decay in the plasma concentration apparently in excess of the actual rate of degradation. However, the renal excretion values indicated extremely low turnover rates.

In one other cirrhotic subject (Gl. H., No. 4) with increasing ascites, the renal excretion also showed lower values than the rate determined from the plasma concentration curve. Although TEA must have been increasing slightly during the period of study, if the net increase in ascitic fluid albumin is calculated as the product of TEA at distribution equilibrium (385 grams) and the difference between the plasma concentration decay rate (2.9 per cent per day) and the degradation rate ($\frac{\text{renal excretion}}{\text{per cent retained}} = 1.8$ per cent per day), a mean value of 4.2 grams per day is obtained. This agrees fairly well with the value (3.6 grams per day) derived by dividing the total albumin (214 grams) removed by paracentesis at the end of the study by the number of days elapsed since the previous tap (59 days). During a period of 80 days prior to the study this patient had been relieved of 585 grams of albumin by repeated paracenteses. The losses (7.3 grams per day) by this route alone were almost as much as the total turnover values in some non-cirrhotic subjects in this group. This indicates the tremendous additional load imposed upon the albumin productive capacities of the liver by repeated withdrawal of ascitic fluid in these subjects. It is conceivable that, in some cases, such practice might demand the synthesis of even greater than normal amounts of albumin.

In most of the studies with the remaining lots, insufficiently long observation periods and the presence of rapidly degraded components combined to produce apparently shorter half time in the plasma curves and poor agreement between rates determined from plasma and urine curves. In most of these cases the turnover rate of the final component as determined from the urine excretions was lower than the rate derived from the plasma concentration curves essentially because the constructed slopes of the terminal portions of the plasma curves were more prejudiced by earlier points which included varying amounts of the faster degraded components. The turnover rates given in Table IB, therefore, are, in general, spuriously high and represent only maximum values.

In some lots the rate of degradation of these faster components was only slightly greater than that of the slowest component (*e.g.*, lot B12–2–2). In others (*e.g.*, lot No. Abbott A 216–2) probably due to a more marked degree of altera-

tion of the protein, extremely rapid breakdown of a fairly large fraction was obvious within the first day or two (see Table IB, column S). Since the slope continued to change for a few weeks, however, it is likely that a more or less continuous spectrum of components degraded at different rates was present. For this reason it is questionable whether a "final component" in these cases was ever really observed. Furthermore, because of inadequate observation periods, the calculated "fraction of faster components" is, in some cases (*e.g.*, Abbott A 014–2), falsely low, owing to a spuriously steep slope in the plasma concentration curve extrapolating back to an apparently high zero time value.

Review of the entire mass of data thus leads to the conclusion that the apparent rates of degradation depended in large part on the particular lot of iodo-albumin used. However, with what were considered the best batches of material, the rates were appreciably less than 5 per cent per day.

Effect of overiodination of albumin on the degradation rate

In order to investigate the possibility that differences in the rates of degradation were related to the degree of iodination of the protein molecules, two portions of iodo-albumin containing an average of 1½ and 11 iodine atoms per molecule of protein, respectively, were prepared identi-

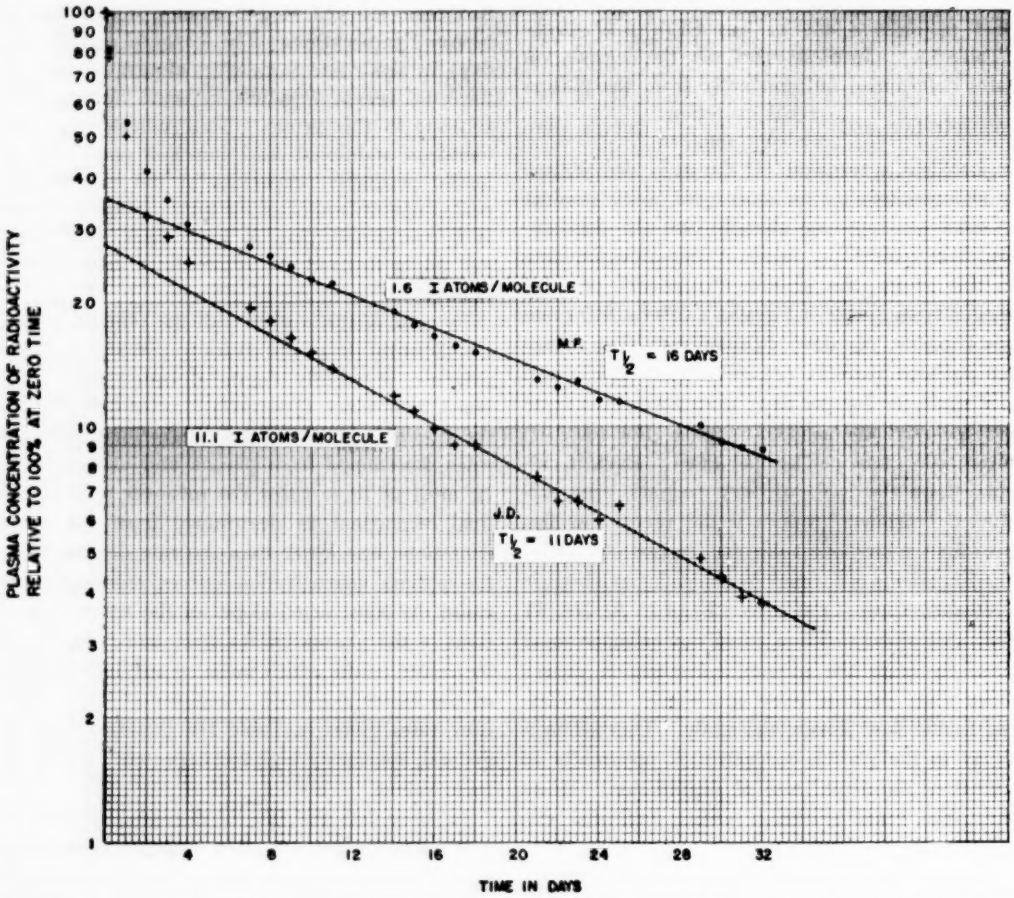


FIG. 10. PLASMA CONCENTRATION CURVES IN SUBJECTS M. F. (NO. 2) AND J. D. (NO. 1) SHOWING RELATIVELY RAPID DEGENERATION IN THE MORE HEAVILY IODINATED PROTEIN

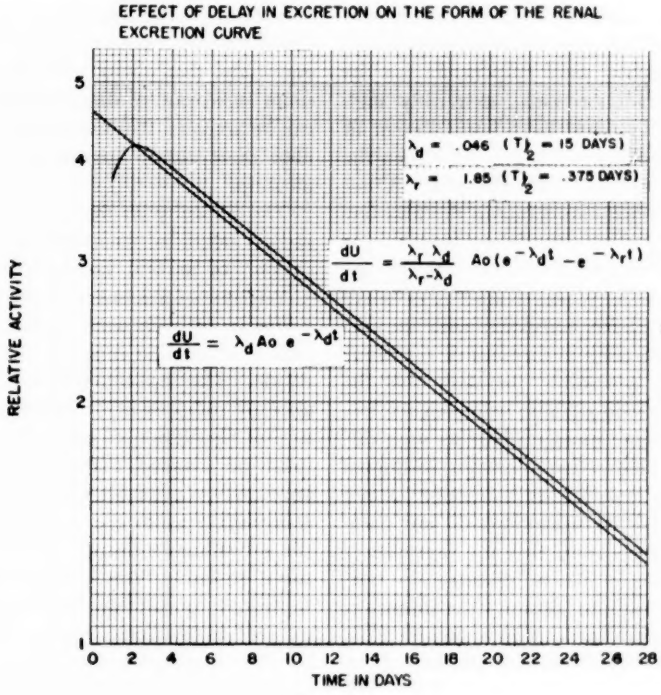

EFFECT OF DELAY IN EXCRETION ON THE FORM OF THE RENAL EXCRETION CURVE
λ_d = .046 ($T_{\frac{1}{2}}$ = 15 DAYS)
λ_r = 1.85 ($T_{\frac{1}{2}}$ = .375 DAYS)
$\frac{dU}{dt} = \frac{\lambda_r \lambda_d}{\lambda_r - \lambda_d} Ao(e^{-\lambda_d t} - e^{-\lambda_r t})$
$\frac{dU}{dt} = \lambda_d Ao\, e^{-\lambda_d t}$
RELATIVE ACTIVITY
TIME IN DAYS
1
2
3
4
5
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28

FIG. 11

See Appendix A for interpretation.

cally [6] from the same batch of protein and each was administered to a non-cirrhotic subject (M. F., No. 2 and J. D., No. 1, respectively). The plasma concentration curves (Figure 10) clearly indicated a difference in the rates of degradation, the more highly iodinated material being degraded more rapidly. Ultracentrifugal analysis revealed marked alterations in the sedimentation of the latter material (Figure 9 e). In spite of these obvious changes, however, the rate of degradation was not inordinately fast ($T_{1/2} = 11$ days) and suspicion of such marked alterations would not have been aroused by the renal excretions (Table IA, column S). Because of the exceptional care taken in the preparation of this lot, fairly uniform iodination of the protein molecules must have been obtained and the material, therefore, behaved biologically in a more or less uniform manner.

In view of the very rapid combination of elemental iodine with protein in the process of iodination, it is quite conceivable that during preparation, some portion of the protein may become very heavily iodinated even when the average iodine : protein ratio is no more than 1 to 2 atoms per molecule. These very heavily iodinated molecules, containing a large portion of the total radioactivity may yet comprise only a very small fraction of the total protein. Thus, alterations in the tagged protein may fail to be detected in the ultracentrifuge. In the case of the albumin labeled with an average of 11 I atoms per molecule, abnormality was readily evident in the ultracentrifugal pattern probably because much of the protein itself must have been altered at such a high average iodination level. Where a low average iodination level was present, as in all the other lots used in this study, it was repeatedly demon-

[6] The iodine solution was slowly added to the albumin solution with continuous mixing. After an amount calculated to produce a ratio of 1.5 I atoms per molecule had been added, one-half of the protein solution was removed and the remainder was then more heavily iodinated in the same fashion. The final ratios as determined from nitrogen analysis were 1.58 and 11.1 I atoms per molecule albumin, respectively.

strated that a normal sedimentation pattern did not insure biological integrity of the tagged moiety.

If uneven iodination were responsible for the variability of degradation rates, extremely high iodine: protein ratios must have been present in some molecules of many of the lots since even the 11 I atoms per molecule iodo-albumin did not lead to such high renal excretions in the first few days as were observed in most of the Abbott preparations. The inference that, in these lots, a large part of the radioactivity was bound to a small moiety of the albumin is consistent with the frequent failure to identify abnormalities on ultracentrifugation. It is possible, therefore, that the apparent multiple iodo-albumin components frequently observed were merely albumins with different degrees of iodination although additional alterations during isolation or iodination cannot be excluded. These considerations do not absolutely negate the possibility that the presence of multiple components in "native" albumin also contributed to these variations, although the absence of more than one component in at least one lot (No. B1-7-3) offers no support for this alternative.

DISCUSSION

The biological behaviour of the isotopically labeled simple ion or chemically defined compound may be accepted, in general, as reflecting that of the native unlabeled species except for consideration of radiation and so called isotope effects. In the case of complex substances such as proteins, however, *in vitro* labeling procedures may induce subtle alteration beyond the scope of detection of available physical and chemical techniques. The validity of the tracer thus remains suspect. Furthermore, its identical behaviour to that of the native substance in certain biological systems (*e.g.*, immunological) does not, in itself, warrant the assumption of such similarities in other systems (*e.g.*, hormonal activity or response to catabolic processes). For example, the precipitation by specific antibody of bacterial toxin and toxoid hardly establishes their identical toxicity. Similarly, the parallel rate of disappearance of I^{131} labeled and native human serum albumin from the blood stream of the rabbit (3), to which they are both foreign proteins, does not necessarily insure similar behaviour in the human subject. Indeed, iodo-albumin is not itself a protein native to man. Conversely, however, it has been repeatedly demonstrated elsewhere (3, 4), as well as in the present study, that obvious alteration does lead to a more rapid rate of disappearance and it probably can be accepted that the slowest rate of disappearance most nearly approaches that of the native albumin.

The difficulty in determining whether a more rapidly degraded component is an altered protein or one of several normal albumin fractions is merely one of a number of problems in the interpretation of such data as are reported here. A principal question concerns the process of degradation. The implication that the rate of disappearance of radioactivity from the body reflects the rate of dissolution of the entire albumin molecule must be avoided. The observations presented here and in available published reports offer no information as to the mechanism of splitting of the I^{131} albumin linkage in the body. Although, more probably, the iodotyrosine fragments are separated by peptide bond hydrolysis, there is, as yet, no evidence against the possibility that simple deiodination alone occurs *in vivo* in this abnormal protein. Even if hydrolysis alone were demonstrated, the rate of this process is not manifestly the same as the turnover of the normal tyrosine fragments. Furthermore, if there is any turnover within the intact protein, then the rates for the various amino acid residues may be quite different. In this event the term "albumin turnover" would be quite meaningless unless qualified by reference to a specific amino acid. Multiple isotope labeling in different amino acid positions may clarify some of these problems.

Where N, C or S isotopes are used it is reasonable to expect an error in the labeled amino acid turnover rates obtained because of reutilization of some fraction of the isotope released by protein degradation. This particular error is probably of no significance in data obtained from I^{131} labeled protein studies, especially in the presence of thyroid blockage. In the absence of thyroid binding, I^{131} administered as iodide (12, 13) or as di-iodotyrosine (14) is almost quantitatively recovered in the urine within 48 to 72 hours. Further evidence is offered by the recovery of all detectable plasma radioactivity in the electrophoretically separated albumin fraction.

The final question concerns possible differences

among the albumins synthesized by the body in health and various disease states, and whether pooled serum albumin is handled in the same fashion as endogenous protein. In cirrhosis and the nephrotic syndrone, Luetscher (15) has demonstrated an alteration in the ratio between the two components of human serum albumin normally demonstrable in the electrophoresis apparatus at pH 4.0. Therefore, isolation and labeling of the subject's own albumin would be a more valid technique, but the accumulation of any considerable mass of data by this method represents a formidable undertaking.

In view of these considerations, it is advisable until further information is available, to interpret the observations presented here, and in similar studies, simply in terms of separation of the radioactive label from the protein, especially when dealing with degradation rates. In the case of distribution phenomena it is more probable that the behaviour of I^{131} labeled albumin closely reflects that of the native protein. The distribution studies have clearly demonstrated that mixing in the entire exchangeable albumin pool is not essentially complete until 4 to 7 days after administration and may be even more prolonged in subjects with massive ascites. Schoenberger, Kroll, Sakamoto, and Kark (16) studied the rate of appearance of I^{131} labeled albumin in the ascitic fluid of a cirrhotic subject in a manner similar to that reported here. They found that equilibrium between plasma and ascitic fluid took 38 and 47 hours in the two studies performed on their patient. However, at the time of the studies, their subject had ascitic fluid volumes of only 890 and 1950 ml., respectively. Their results, therefore, are not inconsistent with our findings of 9 to 10 day equilibration periods in subjects with ascitic fluid compartments of 7 to 19 liters. In fact, the albumin transfer rate across the peritoneal surface was even greater in our subject than in theirs.

Previous studies (3, 16, 17), which have dealt only with observation periods of 11 to 15 days or less, have reported significantly faster degradation rates than in the present study. In general, these rates were calculated from plasma points taken as early as the third day. Inspection of the published curves in these reports indicates that a possible flattening out of the curves has been ignored. Since distribution is usually not complete for 4 to 7 days, inclusion of these early points results in an apparent but erroneous fast rate of degradation. Furthermore, the presence of slower components is likely to escape detection unless observations are carried out for a period of time sufficient to account for the excretion of most of the administered dose.

Although the experiments with heavily iodinated albumin may explain the presence of multiple components with different degradation rates, there is evidence to support the concept of more than one native serum albumin. The presence of two electrophoretically distinct albumin components at pH 4.0 observed by Luetscher (15) has been confirmed by Cohn, Hughes, and Weare (18). According to Hughes (19, 20) more than half of normal serum albumin is mercaptalbumin with a single available sulfhydryl group per molecule, the remaining serum albumins appearing to have no available sulfhydryl groups. The labeling of these various fractions should be of interest.

Whether the multicomponent systems observed in the present study were related entirely to artifacts produced by iodination or in part to different native albumins, the major component in the best preparations was degraded at the slowest rate which approximated a half time of 15 to 23 days. These values are consistent with the results obtained by biosynthetic techniques (1, 2). However, in light of the previous discussion, conclusions based on this similarity in rates must be guarded. The marked variability of degradation rates observed in the different lots of radioiodinated albumin further emphasizes the need for a conservative approach. It is hoped that further studies will be directed along the lines of these inquiries before precarious deductions are formulated on the basis of I^{131} labeled protein "turnover" rates.

SUMMARY AND CONCLUSIONS

1. I^{131} labeled human serum albumin was administered intravenously to 46 subjects in 53 experiments. Plasma, urine, fecal and ascitic fluid concentrations of radioactivity were followed for periods up to six weeks. Various body sites were assayed by *in vivo* techniques.

2. The labeled albumin reached distribution equilibrium in 4 to 7 days except in subjects with massive ascites where this period was prolonged. The apparent space of distribution in non-ascitic

subjects averaged about 2.5 times the plasma volume. Two apparent rates of transfer between plasma and extravascular spaces, with half times of approximately 3 and 24 hours, were observed.

3. Radioactivity released by degradation of the protein-iodine bond was almost completely accounted for by renal excretion in the presence of thyroid blockage.

4. Where observation periods were adequate, independent determinations of degradation rates from plasma and urine data agreed well. Most lots of I^{131} labeled albumin utilized contained variable quantities of rapidly degraded components. The major components in the best lots were degraded at a rate of about 4 per cent per day ($T_{1/2}$ = 17 days), or less, which agrees well with values obtained by biosynthetic methods but not with those previously reported by this method.

5. It is concluded that the validity of the I^{131} labeled albumin as a tracer for the study of endogenous albumin "turnover" rates has not been unequivocally demonstrated. Reasons for the disagreement with results of previous studies along the same lines are discussed.

APPENDIX A

Effect of renal removal of iodide on the renal excretion curve

The I^{131} of fragments released from the degraded protein is probably very rapidly converted to iodide, as has been demonstrated for di-iodotyrosine (14). At normal renal clearance rates, the half time for renal excretion of iodide is about nine hours (21), approximately 84 per cent being excreted in a 24 hour period.

Let

A = quantity of I^{131} tagged albumin in the body,
I = quantity of radioactive iodide in the body,
U = cumulative quantity of radioactive iodide in the urine,
λ_d = rate of degradation of iodo-albumin and release of I^{131} from the protein bond,
λ_r = rate of renal excretion of radioactive iodide,
then

$\frac{dU}{dt}$ = excretion of radioactive iodide per unit time.

Neglecting the very short time for conversion of radioactive iodinated tyrosine to radioactive iodide,

$$\frac{dA}{dt} = -\lambda_d A \qquad (1)$$

$$\frac{dI}{dt} = -\lambda_r I + \lambda_d A \qquad (2)$$

$$\frac{dU}{dt} = \lambda_r I. \qquad (3)$$

Integrating and substituting, we obtain:

$$A = A_0 e^{-\lambda_d t} \qquad (4)$$

$$I = \frac{\lambda_d A_0}{\lambda_r - \lambda_d} (e^{-\lambda_d t} - e^{-\lambda_r t}) \qquad (5)$$

$$\frac{dU}{dT} = \frac{\lambda_r \lambda_d A_0}{\lambda_r - \lambda_d} (e^{-\lambda_d t} - e^{-\lambda_r t}) \text{ (renal excretion curve).} \qquad (6)$$

If all the radioiodine released by iodo-albumin degradation were instantly excreted in the urine:

$$\frac{dU}{dt} = \lambda_d A \qquad (7)$$

substituting (*4*)

$$\frac{dU}{dt} = \lambda_d A_0 e^{-\lambda_d t} \text{ (albumin degradation curve).} \qquad (8)$$

Since λ_r is of the order of 25 to 40 times as great as λ_d even at moderately low renal clearance rates, it is clear, that within a short time, the second term in the brackets of equation (*6*) becomes relatively insignificant and the curves of (*6*) and (*8*) become virtually identical except for slightly different intercepts. Theoretical curves have been plotted for equations (*6*) and (*8*) with half times for renal removal of iodide and albumin degradation of 9 hours (0.375 days) and 15 days, respectively. It is apparent (Figure 11) that, at these rates, the renal excretion curve becomes virtually identical with the curve of iodo-albumin degradation after the first day or so.

APPENDIX B

Effect of multiple components on the characteristics of the plasma concentration and the renal excretion curves

Let P and Q be quantities of different iodo-albumins, p and q, degraded at rates λ_p and λ_q, respectively, where $\lambda_p > \lambda_q$. Then,

$$P = P_0 e^{-\lambda_p t}$$

$$Q = Q_0 e^{-\lambda_q t}.$$

If the apparent space of distribution remains constant, the total amount of iodo-albumin, A, at any time, t, remains constantly proportional to the plasma concentration of iodo-albumin, c. Thus A = kc where k is a constant equal to the apparent space of distribution. Disregard the mixing phase during which the iodo-albumin reaches equal specific activity in all compartments of the albumin pool. Then,

$$A = kc = P_0 e^{-\lambda_p t} + Q_0 e^{-\lambda_p t}. \qquad (1)$$

Assume that all degraded protein radioactivity appears in the urine immediately, which is a good approximation (see Appendix A). Then,

$$u = -\frac{dA}{dt} = \lambda_p P_0 e^{-\lambda_p t} + \lambda_q Q_0 e^{-\lambda_q t} \qquad (2)$$

u = renal excretion per unit time.

Equation (*1*) is essentially the plasma concentration curve and equation (*2*) is the urine excretion curve. Both obviously have the same form being resolved into two exponential terms. The corresponding slopes are identical but have different relative intercepts.

Since $\lambda_p > \lambda_q$,

$$\frac{\lambda_p P_0}{\lambda_q Q_0} > \frac{P_0}{Q_0}.$$

The renal excretion curve, therefore, has a relatively larger first term than the plasma concentration curve. It follows, then, that the renal excretion curve will have a sharper slope at all times than the plasma concentration curve, that this difference will be more marked in the earlier segments and that the approach to a single exponential straight line decay of the terminal segment will be apparent earlier in the plasma concentration curve. These are the very characteristics of the curves observed in these studies.

APPENDIX C

Exchange of iodo-albumin between plasma and ascitic fluid following intravenous administration

Constancy of albumin concentrations, volumes of the various compartments and transfer rates are assumed. Since exchange between plasma and ascitic fluid is rapid compared with the rate of degradation, differences in specific activity in the two compartments resulting from differential degradation in the compartments is neglected.

Let

P' = total iodo-albumin in plasma compartment, p, containing P grams albumin

F' = total iodo-albumin in ascitic fluid compartment, f, containing F grams albumin

Q' = total iodo-albumin in all other extravascular compartments, q, containing Q grams albumin

$P' + Q' = M'$ = total extraperitoneal iodo-albumin.

a) Under the assumption that equilibrium between p and q is rapid as compared with that between p and f, m may be regarded as a single pool in exchange with f. Then,

$$\frac{dM'}{dt} = -\lambda_m M' + \lambda_f F' \qquad (1)$$

$$\frac{dF'}{dt} = -\lambda_f F' + \lambda_m M' \qquad (2)$$

where

λ_m is the rate of transfer of iodo-albumin from m to f

λ_f is the rate of transfer of iodo-albumin from f to m

and at equilibrium

$$\lambda_m M' = \lambda_f F'.$$

Solving equations (*1*) and (*2*) and integrating, we obtain,

$$F' = (M'_0 - M'_E)(1 - e^{-(\lambda_f + \lambda_m)t}) \qquad (3)$$

$$M' = M'_E + (M'_0 - M'_E)e^{-(\lambda_f + \lambda_m)t} \qquad (4)$$

where M'_0 and M'_E are zero-time and equilibrium values, respectively.

In subject H, the ascitic fluid volume was not measured during the period of equilibration. Total ascitic fluid iodo-albumin was estimated as the difference between total exchangeable albumin and 2.5 times total plasma albumin (since the ratio of extravascular to plasma albumin in nonascitic subjects is approximately 60/40). The values obtained for endogenous albumin in the various compartments are then

TEA = 354 grams (Total exchangeable albumin)
P = 110 grams
Q = 165 grams
F = 79 grams.

The ascitic fluid albumin concentration was 1.18 grams per 100 ml. indicating an ascitic fluid volume of 7.5 liters which appeared reasonable.

Since

$$\lambda_m M = \lambda_f F.$$

Then,

$$\frac{\lambda_m}{\lambda_f} = \frac{F}{M} = \frac{79}{275}. \qquad (5)$$

From the ascitic fluid distribution curve (Figure 6) and, equation (*3*)

$$\lambda_m + \lambda_f = .0150 \text{ per hour.} \qquad (6)$$

Solving (*5*) and (*6*) we obtain,

λ_f = .0117 per hour
λ_m = .0033 per hour.

The rate of transfer of albumin across the peritoneal surface in either direction is then

$$\lambda_f F = \lambda_m M = .92 \text{ gram per hour.}$$

b) Under the assumption that plasma albumin is distributing into ascitic fluid alone, the resulting equations derived similarly to (*3*) and (*4*) are

$$F' = (P'_0 - P'_E)(1 - e^{-(\lambda_f + \lambda_p)t}) \qquad (7)$$

$$P' = P'_E + (P'_0 - P'_E)e^{-(\lambda_f + \lambda_p)t} \qquad (8)$$

where P'_0 and P'_E are zero time and equilibrium values, respectively, λ_p is the transfer rate of iodo-albumin from plasma to ascitic fluid and λ_f is the transfer rate of iodo-albumin from ascitic fluid to plasma. Now, if the same values for P and F are taken as in Method a) and solved in the same manner,

λ_f = 0.0087 per hour
λ_p = 0.0063 per hour
$\lambda_f F = \lambda_p P$ = .69 gram per hour.

The true value probably lies between the values obtained by Method a) and Method b). During the period of study there was a net increase in ascitic fluid albumin of approximately 4 grams per day (see text). Thus, the rate for passage from plasma to ascitic fluid was 0.17 gram per hour greater than in the reverse direction.

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OBSERVATIONS CONCERNING THE CLINICAL AND METABOLIC EFFECTS OF CORTICOSTERONE (COMPOUND B) IN RHEUMATOID ARTHRITIS [1]

By WILLIAM S. CLARK, JOSEPH A. BLAIS, AND WALTER BAUER

(From the Medical Clinic, Massachusetts General Hospital; the Department of Medicine, Harvard Medical School; and the Massachusetts Department of Public Health)

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Corticosterone is a naturally occurring adrenal corticosteroid which has been isolated from adrenal gland extracts (1, 2), adrenal perfusates (3, 4) and adrenal vein blood (5). Biosynthesis has made this steroid available for clinical studies. It has been demonstrated to influence carbohydrate, nitrogen, and electrolyte metabolism (6, 7), but to be devoid of antirheumatic effects (8). These studies were undertaken to investigate further its metabolic and antirheumatic properties.

PROCEDURE

Corticosterone was administered by mouth in doses of 100 milligrams every 8 hours for 8 days to one patient with typical, moderately severe rheumatoid arthritis. All treatment was discontinued prior to the experiment with the exception of 20 hours bed rest per day and active bed exercises twice daily. Subjective changes in the arthritis were determined daily by questioning the patient on the degree of fatigue, weakness, aching, stiffness, soreness, and pain at rest. Objective changes were determined daily by inspection and palpation of the involved structures by two observers. Balance studies were performed on constant diet and fluid intake by methods described by Reifenstein, Albright, and Wells (9). Stools were collected in four-day periods, and urines in two-day periods. Oral arterial and venous glucose tolerance tests were carried out during the control period and at the termination of treatment.

Case summary. The patient, a 34 year old male (MGH No. 717532), had enjoyed good health until twenty-seven months before admission, when he developed pain and stiffness of the temporomandibular joints. These symptoms subsided within two weeks. He remained well until twenty-three months before entry, when he developed pain, stiffness, and swelling of the elbows, wrists, fingers, knees, and ankles. His symptoms remained constant until fifteen months before entry, when pain in the hips, shoulders, and cervical spine increased. He had had no chills, fever, cardiac, gastro-intestinal or genito-urinary symptoms. He had had anorexia, weight loss of ten pounds, moderate fatigue, and persistent conjunctivitis.

One year before this study, he was given 150 milligrams of hydrocortisone acetate daily for eight days. At the completion of treatment, he was asymptomatic except for mild aching of both shoulders. However, slight to moderate thickening of both elbows and the right wrist remained. He continued unchanged for seven days after treatment, but on the eighth day pain reappeared. Relapse was complete at the end of four weeks. Nine months before entry he was treated with intramuscular cortisone acetate under identical conditions as hydrocortisone acetate and with similar results. Complete relapse again appeared in four weeks, and his condition remained unchanged to the present study.

Physical examination revealed a thin, tense man with prominent eyes and bilateral pterygii. His temperature was 98.6° F. and blood pressure, 140/90. A grade I apical systolic murmur was present, but cardiac size and rhythm were normal. The liver and spleen were not palpable. A few small lymph nodes were palpable in the axillae.

Examination of the joints revealed slight restriction of motion of the temporomandibular joints and cervical spine. The proximal phalangeal joints of all fingers and the second and third metacarpo-phalangeal joints bilaterally were tender and swollen with small effusions. Both wrists were swollen and tender. The elbows were warm, tender, and swollen with effusions. The fingers, wrists and elbows were limited in motion. The shoulders had normal, but painful, passive motion. The lower extremities were normal except for slight thickening of the knees, limitation of dorsi-flexion of the ankles, and tenderness of the metatarso-phalangeal joints. Small subcutaneous nodules were present over both upper ulnae and both lateral malleoli. Muscle atrophy was pronounced about the shoulder girdles and in the forearms and thighs.

Laboratory findings on admission consisted of a normal urinalysis, a hemoglobin of 16 grams per cent, and total white blood count of 8,500. X-ray examinations of the hands, elbows, shoulders, knees, ankles, and feet showed the bones to be well mineralized. There was some soft tissue thickening about the proximal phalangeal joints. Small cystic areas of rarefaction were seen in some of the carpal bones.

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METHODS

The following methods were used: sodium and potassium —flame photometer (10), chloride—Wilson and Ball (11), nitrogen—Pregl (12), calcium—Fiske and Logan (13), phosphorus—Fiske and Subbarow (14), 17 ketosteroids—Nathanson and Wilson (15), sedimentation rate —Rourke and Ernstene (16), serum glucose—Nelson (17), eosinophils—Randolph (18).

RESULTS

Clinical Results

The changes in the arthritis starting with the control period are shown in Figure 1 by the following scheme. The precontrol base-line is given a value of 100 per cent activity. Shaded areas above this line indicate increased activity, and clear areas below this line, improvement.

The patient remained afebrile throughout the experiment. On the third hospital day aspirin (3 grams per day) was discontinued. During the next three days there was a moderate increase in pain, stiffness, fatigue, and anorexia. A slight increase in swelling of the midphalangeal joints occurred. Symptoms and signs remained constant until the nineteenth hospital day, that is, two days before starting corticosterone, when pain and stiff-

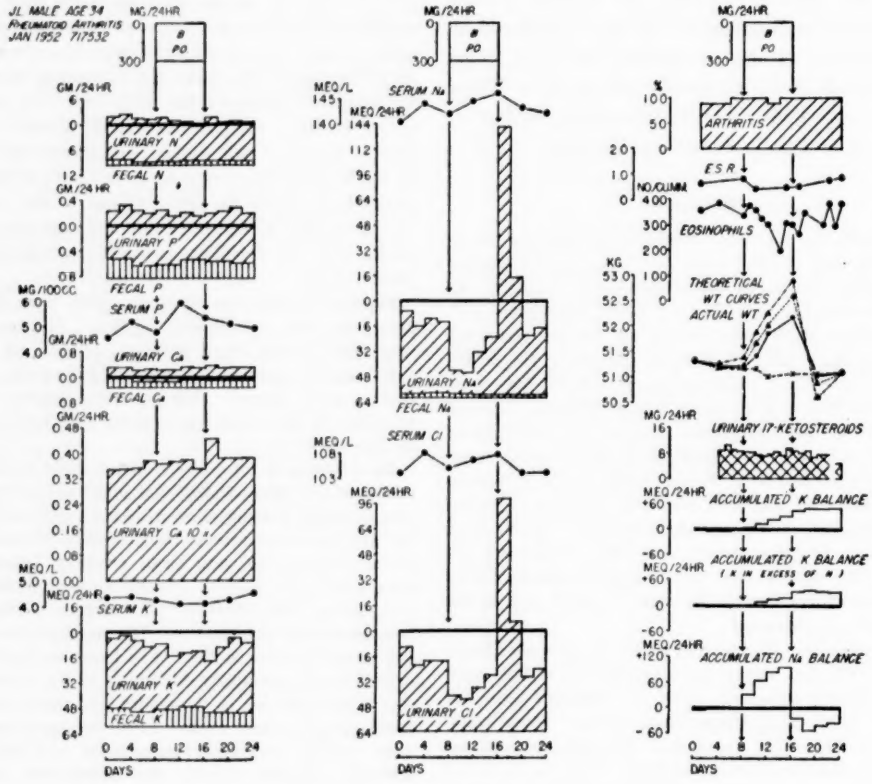

FIG. 1

The first column indicates balance data and serum values for nitrogen, phosphorus, calcium, and potassium and urinary calcium. The second column indicates balances and serum values for sodium and urinary chlorides. The third column indicates the clinical course, sedimentation rates, circulating eosinophils, urinary 17 ketosteroids, and cumulative sodium and potassium balances, including potassium corrected for nitrogen. In plotting theoretical weight curves, the shaded circles indicate actual weight; crosses indicate protoplasm (nitrogen); open triangles indicate protoplasm plus extracellular fluid (sodium); the shaded triangles indicate theoretical weight derived from nitrogen, sodium, and potassium.

TABLE I

Balance data

Periods*	Comp. B mgms.	Na Stool	Na Urine	Na Balance	Cl Urine	Cl Balance	K Stool	K Urine	K Balance	Ca Stool	Ca Urine	Ca Balance	P Stool	P Urine	P Balance	N Stool	N Urine	N Balance
1		4.9	103.9	+ 13.9	106.5	+ 20.3	22.3	87.9	+ 7.2	0.604	0.693	−0.643	0.625	1.50	−0.471	2.87	20.34	−3.69
2		4.9	84.8	++ 33.0	81.2	+++ 45.6	22.3	91.6	++ 3.5	0.604	0.696	−0.646	0.625	1.70	−0.671	2.87	20.73	−4.08
3		6.8	93.1	++++ 22.8	87.2	+++ 39.6	17.0	90.1	++ 10.3	0.393	0.706	−0.445	0.404	1.63	−0.380	1.67	19.97	−2.12
4		6.8	89.4	++++ 26.5	86.9	+++ 39.9	17.0	82.8	++ 17.6	0.393	0.753	−0.492	0.404	1.65	−0.400	1.67	19.55	−1.70
5	600	4.9	30.5	++++ 87.3	43.4	+++ 83.4	19.4	81.9	++ 16.1	0.383	0.733	−0.462	0.417	1.73	−0.493	1.76	19.74	−1.98
6	600	4.9	28.4	++++ 89.4	38.9	+++ 87.9	19.4	68.7	++ 29.3	0.383	0.746	−0.475	0.417	1.53	−0.293	1.76	18.54	−0.78
7	600	3.3	53.9	++++ 65.5	54.3	+++ 72.5	23.0	67.0	++ 27.4	0.540	0.758	−0.644	0.570	1.49	−0.406	2.02	18.54	−1.04
8	600	3.3	72.5	+++ 46.9	69.4	++ 57.4	23.0	69.3	++ 25.1	0.540	0.701	−0.587	0.570	1.38	−0.296	2.02	16.74	+0.76
9		3.9	338.5	− 219.8	291.4	− 164.6	14.9	64.1	++ 38.4	0.490	0.892	−0.728	0.520	1.52	−0.386	2.02	20.76	−3.26
10		3.9	148.8	− 30.1	136.9	− 10.1	14.9	82.3	++ 20.2	0.490	0.767	−0.603	0.520	1.57	−0.436	2.02	18.51	−1.01
11		3.2	73.9	++ 45.6	68.3	++ 58.5	16.7	93.1	++ 7.6	0.497	0.769	−0.612	0.475	1.76	−0.581	1.98	18.89	−1.35
12		3.2	83.8	++ 35.7	77.3	++ 49.5	16.7	87.4	++ 13.3	0.497	0.771	−0.614	0.475	1.56	−0.381	1.98	18.42	−0.88

* Each period represents two days.

ness suddenly increased in both shoulders. Corticosterone was begun on the twenty-first hospital day and continued for eight days. During the first four days of hormone therapy, there was no change in subjective or objective clinical manifestations. On the fifth and sixth days of therapy, the shoulder pain that had appeared two days before treatment subsided, and stiffness and pain generally decreased. On the seventh day, general stiffness and pain and swelling of the midphalangeal joints and wrists increased, and moderate effusions appeared in both knees. His clinical status was unchanged on the eighth day. During the first two days following treatment, knee swelling increased. He was discharged nine days after treatment without further changes.

Laboratory Results

The corrected erythrocyte sedimentation rate (Figure 1) was 0.60 millimeter per minute on two occasions during a two-week period prior to therapy. It ranged between 0.43 and 0.50 millimeter per minute during therapy and between 0.72 and 0.83 millimeter per minute during the recovery period. Synovial fluid was aspirated on the second post-treatment day. Its white cell count was 7,275 cells per cubic millimeter with 71 per cent polymorphonuclear leukocytes, 10 per cent lymphocytes, and 19 per cent monocytes. The mucin precipitated in a soft, stringy clump, leaving a slightly cloudy solution. The fasting synovial fluid sugar was 22 milligrams per cent, while the serum sugar was 61 milligrams per cent. The number of circulating eosinophils were lower during treatment, but a striking decrease appeared in only one of seven determinations (Figure 1).

Results of Balance Studies

Balance data are shown graphically in Figure 1 and tabulated in Table I. Diet values are shown in Table II.

TABLE II

*Results of diet analysis**

Sodium	122.7 mEq.
Chloride	126.8 mEq.
Potassium	117.4 mEq.
Calcium	654.0 mgms.
Phosphorus	1.65 gms.
Nitrogen	19.52 gms.

* Values represent two-day periods.

TABLE III

Theoretical and actual phosphorus balances

Periods	Theoretical P balance	Actual P balance
1	−0.539	−0.471
2	−0.568	−0.671
3	−0.344	−0.380
4	−0.337	−0.400
5	−0.342	−0.493
6	−0.266	−0.293
7	−0.360	−0.406
8	−0.211	−0.296
9	−0.548	−0.386
10	−0.339	−0.436
11	−0.366	−0.581
12	−0.335	−0.381

The following scheme of illustrating actual balance data is used. Intake is plotted downward from a horizontal base-line marked zero, and stool and urine values are plotted upward from the intake line. Shaded areas above the base-line indicate negative balances and clear areas below the base-line indicate positive balances. The ordinate scales for nitrogen, phosphorus, and potassium are chosen on the basis of ratios for protoplasm (9, 19) and bone (9, 20), so that phosphorus balance should equal the sum of the corresponding areas for nitrogen and calcium balances, and potassium balance should equal nitrogen balance. Ordinate scales for sodium and chloride are the same as potassium.

Cumulative electrolyte balances, shown also in Figure 1, were derived by subtracting the control averages from the actual balances and adding the results of each period to the preceding period. The metabolic findings were as follows:

(*a*) *Nitrogen metabolism.* During control periods, the patient was in negative nitrogen balance. Slight nitrogen retention occurred during the administration of corticosterone. Nitrogen excretion increased for two days following treatment, then decreased. Changes in nitrogen excretion thus correlated poorly with administration of the hormone.

(*b*) *Phosphorus metabolism.* Phosphorus excretion decreased slightly during treatment and increased subsequent to treatment. The theoretical phosphorus balances, derived from calcium balances (calcium balance ÷ 2.23) (20), and nitrogen balances (nitrogen balance ÷ 14.7) (19), were in good agreement with actual phosphorus balances, although throughout the experiment actual phosphorus loss exceeded the theoretical phosphorus loss (Table III). Serum phosphorus increased during therapy.

(*c*) *Calcium metabolism.* Urinary calcium remained almost constant during therapy but increased for two days following therapy during a period of diuresis.

(*d*) *Potassium metabolism.* Potassium retention during treatment is shown in both actual and cumulative balance data. Potassium gained during treatment was not lost during the recovery period and could be in part due to sweat losses.

Cumulative potassium balances were also derived after correcting actual potassium balances for actual nitrogen balances (potassium balance—nitrogen balance × 2.7) (21), to estimate potassium gained or lost as intracellular fluid and/or glycogen (9). Although these derived data revealed potassium being retained both in relation to protoplasm and intracellular fluid, or glycogen, some potassium may have been lost in sweat since perspiration noticeably increased during treatment. Serum potassium decreased slightly during therapy.

(*e*) *Sodium and chloride metabolism.* Actual and cumulative balance data revealed sodium retention during therapy and loss during recovery periods. Cumulative data revealed that the post-treatment sodium loss was greater than the gain during treatment. Chloride balances were almost identical to sodium balances. Serum sodium and chloride increased during therapy, but serum chlo-

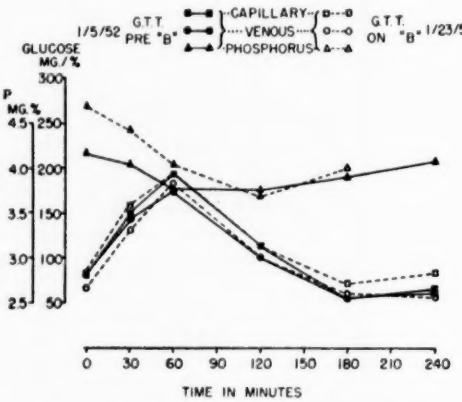

FIG. 2. ARTERIAL AND VENOUS GLUCOSE TOLERANCE CURVES WITH SERUM PHOSPHORUS VALUES

ride did not exceed one unusually high control value.

(*f*) *Body weight.* Changes in actual and theoretical weight, calculated from potassium balances (intracellular fluid), sodium balances (extracellular fluid), and nitrogen balances (protoplasm), by methods described by Reifenstein, Albright, and Wells (9), are shown in Figure 1. Theoretical weight increases, derived from potassium and sodium balances, were greater than actual increases. Sweating, which was noticeably increased during therapy, could account for this discrepancy. The actual weight increase on therapy seemed to relate entirely to fluid retention.

(*g*) *17 Ketosteroid excretion.* Urinary 17 ketosteroid values (shown graphically in Figure 1) decreased slightly during treatment and recovery periods. These changes were not considered significant.

(*h*) *Carbohydrate tolerance.* Arterial and venous glucose tolerance curves during therapy (Figure 2) were not significantly different from control curves. Serum phosphorus decreases following glucose ingestion were approximately the same before and during treatment. There was no glycosuria in daily specimens or during glucose tolerance studies.

DISCUSSION

The appearance of knee involvement with effusions suggested an actual increase in the activity of the rheumatoid arthritis during treatment with corticosterone. Since the knee effusions continued beyond the period of post-treatment diuresis of sodium, they could not be attributed to sodium retention and probably represented a fluctuation in the natural course of the disease. Thus it would appear that corticosterone had neither a favorable nor unfavorable effect on the arthritis.

Retention of sodium and chloride induced by corticosterone in this experiment agreed with the observations of Conn, Fajans, Louis, and Johnson (7). The potassium retention was unexpected and there was no comparable potassium loss during recovery periods. There was some correlation between potassium retention and nitrogen retention which also continued through the recovery periods. All potassium retained could not be explained by nitrogen retention and the unexplained potassium gained could be due to the deposition of glycogen or, possibly, losses in sweat. In favor of the latter explanation are slight discrepancies in the theoretical and actual weight curves and the cumulative loss of potassium unrelated to nitrogen through the recovery period.

Conn, Fajans, Louis, and Johnson have demonstrated that corticosterone prevents hypoglycemia, decreases glucose tolerance, and increases insulin tolerance (7). Similar effects on human carbohydrate metabolism, particularly in patients with Addison's disease, had also been observed by Thorn and Forsham (6). The negative data on carbohydrate activity described in this paper do not invalidate these observations since patients with adrenal insufficiency are more sensitive subjects for evaluation of this metabolic effect.

Like corticosterone, dehydrocorticosterone also has been found to have some carbohydrate activity (6) but no ameliorative effects in rheumatoid arthritis (22), and studies thus far with these two hormones suggest that carbohydrate activity does not endow a steroid with antirheumatic properties. Conversely, no steroid has been found as yet to have antirheumatic properties that does not have carbohydrate activity. Also, cortisone and hydrocortisone are more potent carbohydrate active steroids than corticosterone and dehydrocorticosterone (6), and it is possible that the administration of larger doses of the latter two steroids would favorably influence rheumatoid arthritis. Cumulative evidence suggests that only corticosteroids with active groups at both the carbon 11 and 17 positions have antirheumatic effects.

SUMMARY AND CONCLUSIONS

The administration of corticosterone to one patient with rheumatoid arthritis caused marked sodium and chloride retention but had no antirheumatic effects. No significant changes in carbohydrate tolerance or calcium and nitrogen excretion were evident from the administration of this hormone. Although the data indicated potassium retention, it is doubted that it was a definite hormone effect.

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A FURTHER STUDY OF THE UTILIZATION OF DIETARY GLYCINE NITROGEN FOR URIC ACID SYNTHESIS IN GOUT [1]

By JEAN D. BENEDICT, T. F. YÜ, EDWARD J. BIEN, ALEXANDER B. GUTMAN AND DEWITT STETTEN, JR.

(From the Division of Nutrition and Physiology, the Public Health Research Institute of the City of New York, New York; Department of Medicine, The Mount Sinai Hospital, New York)

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In an earlier report (1) an experiment was described in which a test dose of glycine-N^{15} was fed to a gouty subject and the abundance of N^{15} in uric acid as well as in other urinary constituents was measured for several days. It was found that three times as much of the administered N^{15} could be recovered in urinary uric acid in the gouty subject as in the two normal subjects studied.

The gouty subject used in this initial study had been deliberately selected on the basis of his excessive basal urinary uric acid excretion in order to determine whether increased basal urinary excretion of uric acid, which has been found to occur in about 25 per cent of all gouty subjects studied (2), can properly be ascribed to overproduction of uric acid. In the present report are included similar experiments on three further gouty subjects, one also excreting excessive quantities of uric acid in the urine, the other two excreting amounts within the normal range under the prescribed dietary conditions.

METHODS AND MATERIALS

All subjects were maintained on a constant low purine, restricted protein diet (Table I) for 5 to 10 days before administration of glycine and thereafter throughout the period of urine collections. Estimations of urinary excretion of uric acid and total nitrogen were made during the preliminary and experimental periods.

Isotopic glycine ($N^{15} = 60.5$ atom per cent excess) was fed with breakfast to each subject at a dosage level of 100 mg. per Kg. of body weight. All synthetic, isolation and analytical procedures were conducted precisely as in the earlier experiment. In one subject (A. R.) the magnitude and turnover rate of the miscible pool of uric acid were determined (3) following intravenous injection of 100 mg. of uric acid-1, 3-N^{15}.

[1] This work was carried out with the support of a grant-in-aid from the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health.

Subjects

The gouty subjects selected for study all had unequivocal evidence of the disease, were in about the same (relatively young) age group, had about the same duration of clinical symptoms with similar course, and could not be differentiated on clinical grounds. The one significantly different finding was that H. G. and D. R. consistently excreted unequivocally greater than normal amounts of uric acid in the urine on the indicated diet whereas the urinary urate excretion of A. R. and J. R. consistently remained within normal limits (Table I).

The normal subjects, E. B. and M. R. were described in an earlier report (1). The clinical history of gouty subject D. R. also has been described (1).

H. G., a 38 year old white male, had his first attack of gout at the age of 25. In the past 13 years, acute attacks were never frequent but increased in severity. His mother was stated to have arthritis, but there is no family history of gout. A small tophus in one ear and a punched-out area seen on X-ray at the first left metatarsal joint have been noted. Routine urinalysis was normal; serum NPN, 34 mg. per 100 ml.; inulin clearance, 117 ml. per min. (91.5 ml. per min. after correction for surface area); serum uric acid, 10.0 to 12.0 mg. per 100 ml.

A. R., a 42 year old white male, had his first attack of acute gout at 34. Acute attacks have increased in frequency, occurring weekly or fortnightly prior to November, 1950. He has been on a regimen of colchicine prophylaxis since that time. There are no superficial tophi, but X-ray reveals punched-out areas at the bases of both great toes. Routine urinalysis was normal; serum NPN, 30 to 35 mg. per 100 ml.; PSP excretion, 80 per cent after 2 hours; inulin clearance, 100 ml. per min. (88.2 ml. per min. after correction for surface area); serum uric acid 9.0 to 10.0 mg. per 100 ml.

J. R., a 40 year old white male, had his first attack of gout at age 31. The maternal grandfather and two of nine siblings have had gout. Both parents and five siblings have hyperuricemia. Colchicine was of benefit both therapeutically and prophylactically. No tophi have been found, nor is there evidence of tophaceous deposits on X-ray examination. Routine urinalysis was normal; blood urea nitrogen, 16 mg. per 100 ml.; inulin clearance, 98.5 ml. per min. (87.7 ml. per min. after correction for surface area); serum uric acid, 8.5 to 10.0 mg. per 100 ml.

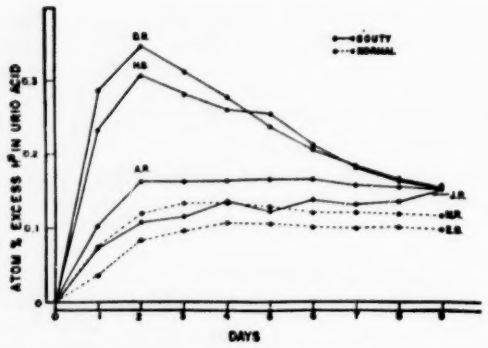

FIG. 1. THE ABUNDANCE OF N^{15} IN DAILY URINARY URIC ACID FROM FOUR GOUTY AND TWO NORMAL SUBJECTS

The concentration of N^{15} as atom per cent excess has been plotted against time in days.

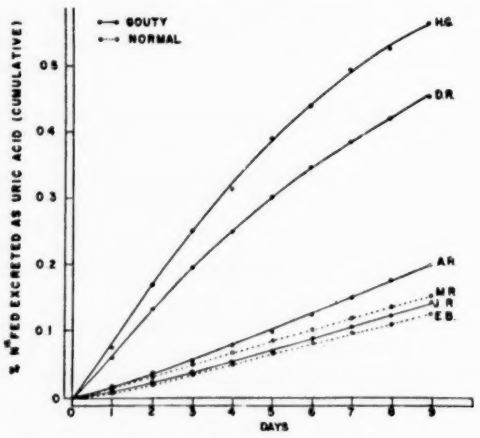

FIG. 2. THE EXCRETION OF N^{15} AS URIC ACID BY FOUR GOUTY AND TWO NORMAL SUBJECTS

The per cent of the total dose of administered N^{15} which was excreted has been plotted cumulatively.

RESULTS AND DISCUSSION

The concentrations of N^{15} in urinary uric acid following the ingestion of a single test dose of glycine-N^{15} are plotted as a function of time in Figure 1. In two of the gouty subjects, D. R. and H. G., far higher concentrations of isotope are found than in the normal control experiments (M. R. and E. B.); the initial rise is more rapid as is also the subsequent decline in N^{15} abundance. Maxima are obtained in these two cases on the second day, where in the normals, maxima appear on the third or fourth day. Subject A. R. excreted uric acid which may be somewhat more enriched with N^{15} than normal. Subject J. R., on the other hand, gave normal values for the first few days after feeding of glycine-N^{15} but, in contrast to the normal subjects, the uric acid excreted by this subject continued to show progressive enrichment with N^{15} up to the ninth day. The experiment upon this last subject was repeated and essentially superimposable data were obtained.

From the quantity of N^{15} fed, the quantity of uric acid excreted in each day's urine and the N^{15} concentration in this uric acid, the percentage of the administered dose of N^{15} excreted as uric acid has been calculated and this information is plotted

TABLE I

Per cent of dose of administered N^{15} excreted as uric acid and as total nitrogen over a nine day period

Case	Wt. kg.	Daily dietary intake*					Daily excretion		Per cent of N^{15} fed excreted as	
		Protein		Fat	COH	Calories	Total N	Uric acid N	Total N	Uric acid N
		g.	g./kg.	g.	g.		g.	mg.		
Normal										
E. B.†	76	48	0.6				7.6 ± 0.5‡	131 ± 4§	33.0	0.125
M. R.†	80	80	1.0				11.1 ± 0.8	159 ± 8	45.4	0.153
Gouty										
H. G.	104	75	0.7	45	580	3,025	11.9 ± 1.0	334 ± 36	50.9	0.563
D. R.†	90	68	0.8	67	340	2,235	10.8 ± 0.4	228 ± 6	44.6	0.453
A. R.	85.5	50	0.6	70	205	1,645	8.0 ± 0.4	144 ± 14	39.7	0.198
J. R. (1)	84.5	59	0.7	67	200	1,640	9.4 ± 0.2	131 ± 5	41.2	0.142
J. R. (2)	89	74	0.8	65	205	1,700	11.8 ± 0.7	134 ± 10	46.5	0.130

* Diet limited to cereals and other grain products, milk and milk products, eggs, non-leguminous vegetables and fruits; no meats, fish or fowl.

† From previous report (1).

‡ Average deviation.

§ Mean urinary uric acid N excretion of 13 normal subjects on basal diet: 139 ± 23 mg. per 24 hr.

cumulatively in Figure 2. It will be seen that at all times gouty subjects H. G. and D. R. had eliminated a larger fraction of administered glycine nitrogen as uric acid than had the normal subjects. Subject A. R. certainly did not deviate markedly from normal in this regard, whereas subject J. R. appears to fall within the normal range.

In Table I are given the results of accumulated excretions over the nine-day period following feeding of glycine-N^{15}. The normal subjects excreted 0.12 to 0.15 per cent of the ingested N^{15} as uric acid in this time interval. Subject J. R., studied on two occasions, excreted 0.13 to 0.14 per cent, whereas subject A. R. excreted 0.20 per cent of administered glycine nitrogen as uric acid. A far higher fraction, three to four times normal, was found in the two subjects H. G. and D. R., who excreted 0.56 and 0.45 per cent, respectively, of dietary glycine nitrogen as urinary uric acid.

In two of the patients studied (H. G. and D. R.) there is unequivocal evidence for abnormally rapid incorporation of dietary glycine nitrogen into uric acid. In the remaining two patients this effect is either unimpressive or absent. A good rank order correlation is to be noted between the daily excretion of uric acid in the urine and the per cent of ingested isotope appearing in urinary uric acid (see Table I). It would seem as though patients suffering from gout may crudely be divided into two types, those who synthesize uric acid abnormally rapidly and, given normal renal function, are prone to excrete large amounts of uric acid in the urine, and those who are within normal limits in these two regards. Muller and Bauer have recently described a case belonging to the latter category (4). Whether this difference results from an actual difference in the pathological process or whether it represents the same process at different stages remains to be determined.

The possibility was entertained that the gouty patients whose uric acid was not isotopically enriched above that of the normal subject might also be overproducing uric acid. Such overproduction might not be apparent in the isotope values if the miscible pool of uric acid were excessively large. A very large miscible pool of preformed uric acid would dilute the isotope in the newly synthesized uric acid. The magnitude of the miscible pool of uric acid in patient A. R. has therefore been determined (3) and was found to be 1,500 mg. (normal value, about 1,100 mg.). This value was not considered to deviate sufficiently from normal to permit the conclusion that any impressive overproduction of uric acid was occurring in this patient.

SUMMARY

Glycine-N^{15} has been fed in a standard experiment to gouty subjects maintained on a purine-poor, restricted protein diet and the excretion of N^{15} in uric acid has been studied.

In subjects who exhibited abnormally high basal uric acid excretions, an abnormally rapid incorporation of dietary glycine nitrogen into uric acid occurred. In others whose basal excretion was approximately normal, no such evidence of accelerated uric acid synthesis was demonstrable.

The significance of these results is discussed.

ACKNOWLEDGMENTS

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THE RELATION OF DIETARY NITROGEN CONSUMPTION TO THE RATE OF URIC ACID SYNTHESIS IN NORMAL AND GOUTY MAN [1]

By EDWARD J. BIEN, T. F. YÜ, JEAN D. BENEDICT, ALEXANDER B. GUTMAN, AND DEWITT STETTEN, JR.

(From the Division of Nutrition and Physiology, the Public Health Research Institute of the City of New York, Inc., New York; Department of Medicine, The Mount Sinai Hospital, New York)

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In earlier studies the rate of incorporation of dietary glycine nitrogen into uric acid was measured in normal and gouty men (1, 2). The experimental procedure employed was to add a fixed quantity (100 mg. per Kg. of body weight) of glycine-N^{15} to the diet and to determine both the quantity of urinary uric acid and its isotope content during the succeeding time intervals.

In view of the increase in urinary uric acid excretion which follows increased consumption of dietary protein, it has been deemed advisable, in some clinics, to restrict the protein intake in gout. It was considered of interest to explore the possible relationship between the level of protein in the diet and the rate of incorporation of dietary glycine nitrogen into uric acid in order to ascertain whether an increase in the rate of uric acid synthesis was indeed demonstrable when the consumption of dietary protein was enhanced.

MATERIAL AND METHODS

One normal (E. B.) and one gouty subject (D. R.) were selected for this study. Both have already been described (1), the gouty subject being known to excrete excessive quantities of uric acid in the urine and to incorporate glycine-N^{15} at an augmented rate when maintained on a low protein diet (1).

Two experiments were conducted on each subject, the first while on a relatively low purine diet (2), the second on the same diet fortified with skimmed milk powder (Lonalac) to increase the protein intake significantly (Table I) without material increase in fat, carbohydrate or total calories. A suitable interval, of 10 to 12 months, was allowed to elapse between experiments on each of these subjects in order to ensure absence of detectable isotopic enrichment after the initial feeding of N^{15}-labeled glycine, as was demonstrated in each instance. The subjects were maintained on their respective diets at least one week before the dose of glycine-N^{15} was administered.

The same dosage of glycine-N^{15} was used in all experiments (100 mg. per Kg. body weight). All of the synthetic and analytical methods employed were those previously described (1, 2).

RESULTS

Despite the fact that the isotopic glycine must have undergone somewhat greater dilution by non-isotopic glycine prior to absorption from the intestine when the diet was fortified with protein, the uric acid excreted in the early period by both of these subjects was richer in isotope when they were on a high protein regimen (Table I, Figure 1). This indication that incorporation of glycine

TABLE I

Per cent of dose of administered N^{15} excreted as uric acid and as total nitrogen by a normal (E. B.) and a gouty (D. R.) subject over a nine day period on a relatively low and relatively high protein diet

Subject	Protein intake g.	Daily excretion		Per cent of N fed excreted as		Per cent of excreted N^{15} in uric acid (100 B/A)
		Total N g.	Uric acid N mg.	Total N (A)	Uric acid N (B)	
E. B. (1)	48	7.6 ± 0.5*	131 ± 4	33.0	0.125	0.38
E. B. (2)	84	13.4 ± 0.8	193 ± 20	52.1	0.195	0.37
D. R. (1)	68	10.8 ± 0.4	228 ± 6	44.6	0.453	1.02
D. R. (2)	108	17.3 ± 1.3	323 ± 27	52.1	0.603	1.16

* Average deviation.

[1] This work was carried out with the support of a grant-in-aid from the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health.

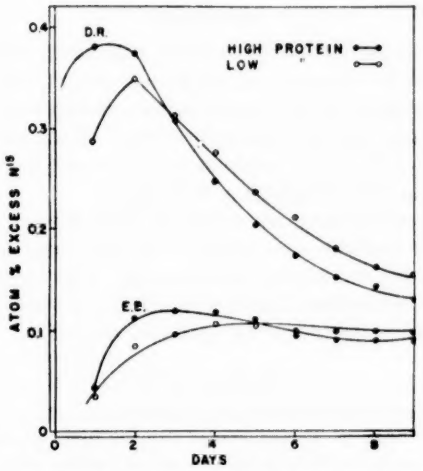

FIG. 1. THE ABUNDANCE OF N^{15} IN DAILY URINARY URIC ACID FROM A NORMAL AND A GOUTY SUBJECT

The concentration of N^{15} as atom per cent excess has been plotted against time in days. Each subject was studied twice, first while on a low protein diet, then while on a high protein diet.

nitrogen into uric acid is more rapid when on a high protein diet is confirmed by the more rapid decline in isotope concentration in uric acid in the latter period of the experiment. At this time the glycine absorbed from the intestinal tract is, of course, predominantly glycine-N^{14}.

These data, together with total N^{15} excretion data, have also been plotted as cumulative percentages of the N^{15} fed which was excreted as total urinary nitrogen and as uric acid (Figures 2 and 3). It will be noted that in both subjects an appreciably greater fraction of the administered glycine nitrogen was excreted in the total nitrogen

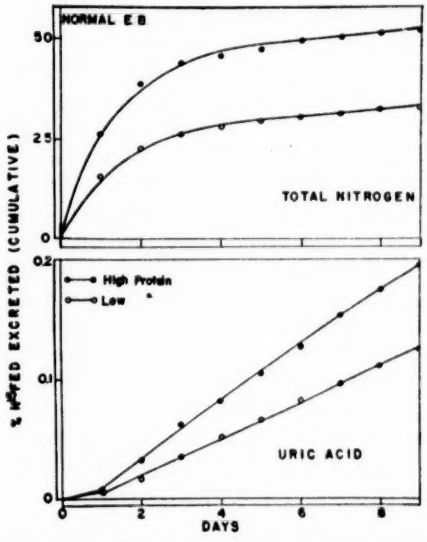

FIG. 2. THE EXCRETION OF N^{15} AS URINARY TOTAL NITROGEN AND URIC ACID BY A NORMAL SUBJECT ON A HIGH AND ON A LOW PROTEIN DIET

The percentage of the total dose of administered N^{15} that was excreted has been plotted cumulatively.

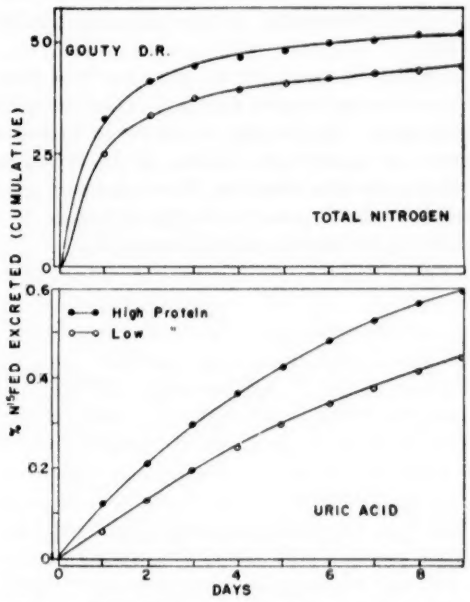

FIG. 3. THE EXCRETION OF N^{15} AS URINARY TOTAL NITROGEN AND URIC ACID BY A GOUTY SUBJECT ON A HIGH AND ON A LOW PROTEIN DIET

The percentage of the total dose of administered N^{15} that was excreted has been plotted cumulatively.

of the urine when a high protein diet was fed. This increase in total N^{15} excretion produced by dietary change is roughly paralleled by the increase in uric acid-N^{15} excretion. Indeed, in the normal subject (E. B., Table I) the per cent of excreted N^{15} which was found in uric acid appeared to be independent of the quantity of protein ingested. A change in protein intake which almost doubled the total urinary nitrogen excretion produced no significant alteration in this percentage (0.38 and 0.37). In the gouty subject (D. R.) a

considerably larger per cent of the excreted N^{15} was recovered in uric acid (1.02 and 1.16 on low and high protein diets). It is questionable whether the change of diet had any significant influence on the percentage in this instance.

When on a diet fortified with protein, both the normal and the gouty subject incorporated dietary glycine nitrogen, and presumably other dietary constituents, more rapidly into uric acid than when on a protein-poor diet. They also apparently delivered nitrogen from glycine to urea more rapidly when the diet was protein-rich and these two processes were accelerated in approximately parallel fashion.

It is hardly necessary to point out that these observations are too few to permit of any definitive conclusions. The present results would, however, appear to support the validity of restriction of dietary protein in situations, like tophaceous gout, where it would appear desirable to reduce to a minimum the rate of uric acid synthesis.

SUMMARY

Glycine-N^{15} has been fed in a standard experiment to one gouty and one normal subject, and the abundance of N^{15} in total urinary nitrogen and in urinary uric acid has been studied. Each subject was studied first while on a low protein diet and again while on a high protein diet.

Both in the normal and in the gouty subject, uric acid synthesis was found to be accelerated when the diet was fortified with protein. This acceleration was approximately paralleled by an acceleration in other metabolic pathways of glycine.

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STUDIES ON THE INTERDEPENDENT EFFECTS OF STRESS AND THE ADRENAL CORTEX ON CARBOHYDRATE METABOLISM IN MAN [1]

By THOMAS W. BURNS,[2] FRANK L. ENGEL, ALBERTO VIAU,[2] JAMES L. SCOTT, JR.,[2] DOROTHY R. HOLLINGSWORTH [2] AND EMILE WERK [3]
WITH THE TECHNICAL ASSISTANCE OF EVANGELINE ROBERTSON

(From the Departments of Medicine and Physiology, Duke University, Durham, N. C.)

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It is now well established that the adrenal cortex is involved in some fundamental manner in the metabolic response to injury and illness, but the precise mechanism by which this gland exerts its influence remains clouded. Originally, based on the observations that certain of the metabolic responses to injury do not occur in the absence of the adrenal cortex, and on the evidence of increased secretion of adrenal steroids during stress, it seemed reasonable to interpret the metabolic changes which occur after stress as direct manifestations of hypersecretion of adrenocorticotrophic hormone (ACTH) and adrenocortical steroids (1, 2, 3). The demonstration that overdosage with ACTH or cortisone produced metabolic changes comparable to those after stress made such an interpretation even more attractive. However, as investigation in this area has been pursued, it has become increasingly apparent that the concept described above is an oversimplification and needs modification. Ingle, in a brilliant series of studies, beginning in 1943, has been chiefly responsible for demonstrating, in animals at least, that the adrenal cortex is not directly responsible for the changes under consideration, although adrenocortical hormone must be present for certain of the changes to take place (4, 5, 6, 7, 8). Thus, the adrenalectomized animal exhibits a normal metabolic response to injury if maintained with an amount of adrenocortical hormone which itself does not produce overdosage effects. Under such circumstances, no increase in adrenal activity is possible, and hence adrenocortical hormone may be considered as necessary but not responsible for certain of the observed metabolic and morphologic alterations after stress. Although the original interpretation of a direct participation of the adrenal cortex in the metabolic response to injury is still widely held, most investigators in this field, including Selye and others who were responsible for the concept, are now in agreement that the role of the adrenal is an indirect one (9, 10).

In this laboratory, attention has been directed for some years to the problem of the nature of the interrelationship between the adrenal cortex and stress in metabolism (11, 12, 13). Ingle's observations have been confirmed, using different techniques. Evidence has been adduced in the rat to indicate that hypersecretion of adrenal steroids is not necessary to initiate the metabolic response to injury, although a high level of secretion may be necessary to sustain it. Thus, it was found that even large doses of ACTH, infused intravenously into the fasted intact rat, produced only delayed effects on nitrogen metabolism, whereas stressful stimuli were followed by immediate changes. It was further shown that if adrenocortical extract is administered to rats subjected to a subthreshold stress, *i.e.*, one which induced either no or only a delayed increase in protein catabolism, the animal responds with an immediate increase in nitrogen metabolism, as if it had been subjected to a more severe stressful stimulus. These studies have led to the concept that the adrenal hormone in some manner permits the organism to respond metabolically to stressful stimuli, the magnitude of the response being dependent primarily on the stress stimulus unless an excess of adrenal hormone is present. In these terms, the so-called "overdosage effects" of exogenous adrenal steroids conceivably could be interpreted as over-responses to minor stressful stimuli, which under ordinary

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[2] Research Fellow in Medicine.

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circumstances, would have no measurable impact on metabolic processes. At the moment, of course, there is no positive proof for such a concept. The present study was designed to extend these observations to one parameter of metabolism in man, namely carbohydrate tolerance. For this purpose, studies were made of the influence of cortisone acetate on the carbohydrate tolerance of normal healthy medical students as compared with ill patients in the hospital. It was found, in confirmation of the views developed from the animal studies, that subjects under the acute or chronic stress of organic illness exhibited a greater change from normal in glucose and insulin tolerance after a given dose of cortisone acetate than did healthy medical students similarly treated.

MATERIALS AND METHODS

The subjects for these studies were 143 normal medical and graduate students and 53 patients. Most of the latter group suffered from organic disease, although a few with severe neuroses were included. With the exception of the special diet control noted below, the students all ate *ad libitum* at the university dining halls. A dietary survey of a representative group of students indicated that the range of diet consumed by them ranged from 1900 to 3000 calories a day with from 200 to 300 grams of carbohydrate, the majority of students consuming diets containing between 2,500 and 3,000 calories. The composition of the dining hall diets was thus not notably different from the ward diet offered the patients, *i.e.*, 2,900 calories, with 110 grams protein, 290 grams of carbohydrate and 140 grams of fat. The tolerance tests were performed on the students during the morning, after they had been sitting through the hour lecture period. Activity during the two to three hours required for the tolerance tests consisted of the walking about necessary to perform routine experiments in Physiology.

The ill subjects were selected as a cross-section of the ward population with organic diseases with the exception of four subjects with anxiety states or hysteria. The latter did not respond differently from those with organic disease and hence were included. No patient with a disease known specifically to modify carbohydrate tolerance, such as diabetes, liver disease or an endocrine disorder, was included. Patients with a family history of diabetes were excluded. Most patients were ambulatory to varying degrees and where possible were not kept strictly in bed during the tests so that their activity would be comparable to the student subjects.

The tolerance tests employed were a standard oral glucose tolerance test, using 1 gram of glucose per kilogram body weight, a glucose-insulin tolerance test modified from Fraser, Albright, and Smith (14), in which 0.1 unit of regular insulin per kilogram body weight was administered intravenously simultaneously with the glucose by mouth (1 gram per kilogram body weight), and insulin tolerance tests, using $\frac{1}{10}$, $\frac{1}{40}$, $\frac{1}{60}$, and $\frac{1}{80}$ unit of regular insulin per kilogram body weight intravenously. All subjects received 50 grams of glucose by mouth ten hours prior to the test in order to make the period of fast constant and to attempt to approximate an intake of carbohydrate of 300 grams or more on the day prior to the test. The insulin used was free of hyperglycemic factor. In order to minimize error in dosage, the insulin was diluted 1:20 with normal saline to increase the volume of the injected solution. When cortisone was given, it was taken by mouth as the crystalline suspension to assure rapid absorption four hours prior to tolerance tests in the acute experiments and every six hours around the clock in the chronic experiments. In general, the dosage used in the acute experiments was 200 mg. as a single dose and in the chronic experiments a total dose of 200 mg. per day was given, 50 mg. every six hours.

Blood was drawn at appropriate intervals into heparinized syringes, and placed in tubes containing potassium fluoride. Protein-free filtrates were prepared within a few minutes, using a copper-tungstate filtrate. Blood for glucose was analyzed by the Somogyi method (15). In the statistical analyses, comparisons were made between increments and decrements in blood sugar levels from zero time as well as between the absolute values for the points on the curves. A probability of $< .05$ was considered probably significant, one of $< .02$ or less definitely significant.

RESULTS

Control studies

To assess the effects of acute administration of cortisone acetate by mouth, three groups of ten normal subjects served as controls. One group received no hormone and the other two received by mouth 200 and 400 mg. of cortisone acetate, respectively, four hours prior to withdrawal of the initial blood samples. Blood was collected for glu-

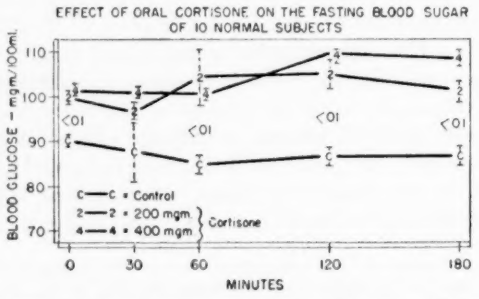

FIG. 1. ORAL INGESTION OF 200 OR 400 MG. OF CORTISONE ACETATE RESULTS IN A SIGNIFICANT ELEVATION OF THE FASTING BLOOD SUGAR FOUR HOURS LATER AND LASTING AT LEAST THREE HOURS

cose determination at 0, 30, 60, 120, and 180 minutes. Figure 1 illustrates that ingestion of cortisone was followed by a significant ($p < .01$) and sustained increase in the fasting blood sugar during the period of four to seven hours after hormone treatment. The blood sugar levels of the untreated subjects showed a small, but statistically invalid, fall during the three hours observation, while those of the cortisone treated subjects exhibited a similarly insignificant tendency to rise. There was no significant difference between the response to 200 and to 400 mg. of cortisone.

When compared with the mean of the two pretreatment days, the fasting blood sugar levels of seven normal subjects receiving 50 mg. of corti-

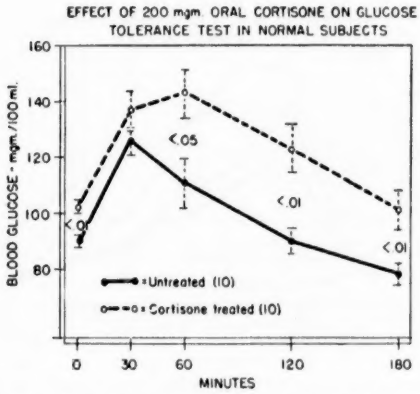

FIG. 2. SIGNIFICANT ALTERATION IN THE ORAL GLUCOSE TOLERANCE TEST OF NORMAL SUBJECTS FOUR HOURS AFTER INGESTION OF 200 MG. OF CORTISONE ACETATE

sone acetate every six hours for eight days were significantly elevated as measured on the third and sixth day of treatment but had fallen to normal again the eighth day. The mean values and standard errors were as follows: Day − 2, pretreatment, 88 ± 2.5; day 0, 84 ± 4.3 (mean pretreatment 86 ± 1.6); day 3 of treatment, 96 ± 4.1; day 6 of treatment, 99 ± 3.2; day 8 of treatment, 89 ± 3.9; day 1 after treatment, 83 ± 2.1; day 4 after treatment, 83 ± 2.2.

Glucose tolerance tests

Glucose tolerance tests were performed on two groups of ten subjects each, one untreated and the other pretreated with 200 mgm. of cortisone acetate orally four hours prior to the test.

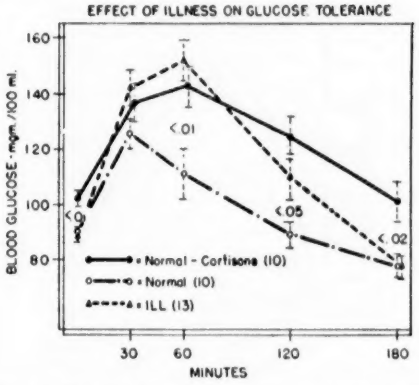

FIG. 3. COMPARISON OF THE IMPAIRMENT IN GLUCOSE TOLERANCE OF NORMAL SUBJECTS RECEIVING 200 MG. OF CORTISONE WITH THAT OF ILL SUBJECTS.

Note that the 0 and 180 minute blood sugar levels are normal in the ill subjects but elevated in the cortisone treated normal individuals compared with the untreated controls.

The results exhibited in Figure 2 demonstrate clearly that blood sugar levels are significantly higher at all points in the tolerance curve of the treated subjects with the exception of the thirty minute point.

When glucose tolerance tests on thirteen ill patients were compared with those on ten untreated normal subjects and ten cortisone treated normal subjects (Figure 3), a clear difference between the effects of cortisone and illness is apparent. Ill subjects showed a definite impairment of glucose tolerance at the 60 and 120 minute points but not at 0, 30, and 180 minutes. This contrasts with the effect of cortisone on the healthy subjects where, as indicated in Figure 2, significant differences from normal are detectable at all times except 30 minutes after glucose.

When cortisone is administered to ill subjects, the impairment of glucose tolerance is strikingly greater than that resulting from illness alone (Figure 4) or from the administration of cortisone to healthy subjects (Figure 2). In this experiment, thirteen ill subjects had glucose tolerance tests performed before and eight of the same group after a single dose of cortisone by mouth. The data of Figures 3 and 4 demonstrate a clear difference between the response of normal and ill subjects to cortisone. Not only is the tolerance curve of the ill group receiving cortisone significantly higher

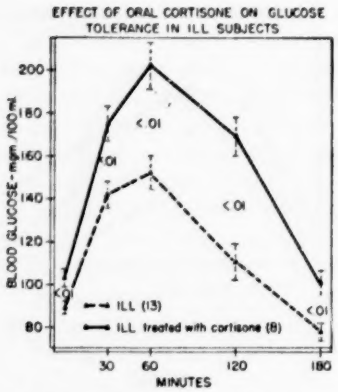

FIG. 4. MARKED IMPAIRMENT OF GLUCOSE TOLERANCE IN ILL SUBJECTS PRETREATED WITH CORTISONE FOUR HOURS BEFORE THE TEST

Compare with Figures 2 and 3.

throughout than that of the ill group not receiving cortisone, but it is also significantly higher at all but the first and last points than that of the healthy subjects receiving cortisone.

Although the patients were offered a diet adequate in calories and carbohydrate, there was no assurance or check that they consumed their entire ration. Since it is well known that impairment of carbohydrate tolerance occurs during carbohydrate restriction in the diet (16, 17), a control study was done to eliminate the possibility that dietary deficiency might account for the different response to cortisone noted above. Accordingly, seven normal students were given trays on the ward containing one half portions of the regular ward diet, *i.e.*, 1,400 calories and 150 grams of carbohydrate per day. It was felt that this would correspond reasonably well with the diets consumed by the ill patients with poor appetites. At the end of seven days, a glucose tolerance test was performed on each subject, preceded by 50 grams of glucose the night before, as in the previous tests. Three subjects were pretreated with cortisone (200 mg., four hours before the tolerance test) and four were not. The diets were continued and at the end of the second week the tolerance tests were repeated, four subjects receiving cortisone and three not. The tolerance curves obtained were then compared with those obtained from normal subjects eating *ad libitum* (Figure 5). Restriction in diet alone caused slight impairment in glucose tolerance, apparent at 30 minutes ($p < .05$). Impairment of carbohydrate tolerance after cortisone pretreatment was approximately equivalent in the group with regulated diet as compared with the group whose diet was not rigidly controlled. The curve of the diet-restricted group was not at all comparable to the curve found in the ill patients receiving cortisone (Figure 4). These results make it seem unlikely that the previous findings are explicable on the basis of dietary variations alone.

Glucose-insulin tolerance tests

In order to explore further the relationship between the effects of cortisone and illness on carbohydrate tolerance, studies comparable with those just described were done with the glucose-insulin tolerance test. This test was done in order to determine whether insulin would modify the altered response to glucose noted after cortisone pretreatment or illness.

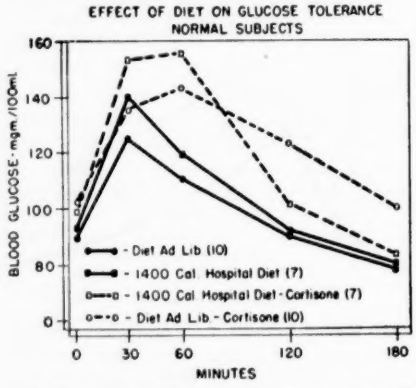

FIG. 5. EFFECTS OF DIETARY RESTRICTION ON CARBOHYDRATE TOLERANCE WITH AND WITHOUT CORTISONE PRETREATMENT IN NORMAL SUBJECTS

As is apparent in Figure 6, curves A, B, and C, four hours pretreatment of normal subjects with either 200 or 400 mg. of cortisone acetate by mouth did not prevent the normal fall in blood sugar following simultaneous administration of insulin and glucose. As in previous experiments, fasting blood sugar levels were significantly higher after cortisone ($p < .01$) and reached higher levels at two and three hours but the latter were not significant statistically. On the other hand, when the nor-

mal subjects were treated with 200 mg. of cortisone daily for six days prior to testing, no fall in blood sugar occurred after insulin and glucose, and except for the 3 hour point, all points on the cortisone curve were significantly higher than those of the untreated controls.

When this same test was performed in ill subjects, with and without cortisone pretreatment (Figure 7), the response was strikingly different from that obtained in normal subjects, untreated or treated acutely or chronically with cortisone. No hypoglycemia developed in either of the ill groups, and significantly higher levels were achieved at 1, 2, and 3 hours than in healthy subjects untreated or receiving a single dose of corti-

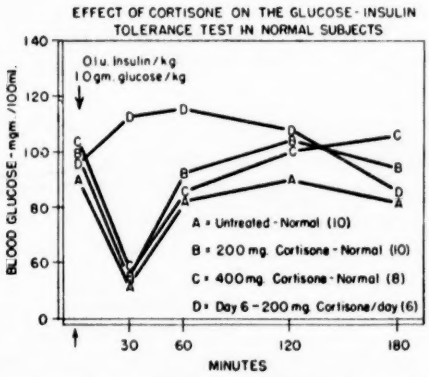
EFFECT OF CORTISONE ON THE GLUCOSE - INSULIN TOLERANCE TEST IN NORMAL SUBJECTS
0.1 u. insulin/kg.
1.0 gm. glucose/kg
BLOOD GLUCOSE - mgm. /100ml.
MINUTES
A = Untreated - Normal (10)
B = 200 mg. Cortisone - Normal (10)
C = 400 mg. Cortisone - Normal (8)
D = Day 6 - 200 mg. Cortisone/day (6)

FIG. 6. LACK OF EFFECT OF 200 OR 400 MG. OF CORTISONE ON THE GLUCOSE-INSULIN TOLERANCE TEST FOUR HOURS LATER AS COMPARED WITH IMPAIRED TOLERANCE ON THE SIXTH DAY OF TREATMENT WITH 200 MG. OF CORTISONE IN NORMAL SUBJECTS

sone. At two and three hours, blood sugar levels in the two ill groups were significantly higher than normals on the sixth day of cortisone treatment. Although the cortisone treated ill group had blood sugar levels at all but thirty minutes higher than the untreated ill subjects, only the difference at fasting met the test of statistical significance. Samples drawn at 1, 2, and 3 hours showed a greater variation and, hence, larger standard error than in the previously described glucose tolerance tests. It is possible that if a larger group of subjects had been tested, the same differences between the two ill groups might have been significantly different, as was the case with the glucose tolerance tests.

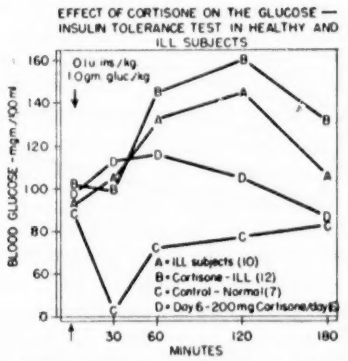
EFFECT OF CORTISONE ON THE GLUCOSE — INSULIN TOLERANCE TEST IN HEALTHY AND ILL SUBJECTS
0.1 u. ins./kg.
1.0 gm. gluc./kg
BLOOD GLUCOSE - mgm./100 ml
MINUTES
A = ILL subjects (10)
B = Cortisone - ILL (12)
C = Control - Normal (7)
D = Day 6 - 200 mg Cortisone/day (6)

FIG. 7. COMPARISON OF RESPONSE IN NORMAL AND ILL SUBJECTS TO GLUCOSE AND INSULIN WITH AND WITHOUT ACUTE OR CHRONIC CORTISONE PRETREATMENT

Insulin tolerance tests

In the past, much emphasis has been placed on the possibility that the impairment of carbohydrate tolerance, which has been reported during stress and in animals which have been treated with adrenal steroids, is due to a peripheral blocking of insulin action by adrenal steroids. This concept is supported by the experiments of Ingle in which it was found that force-fed rats which were made diabetic by the administration of very large doses of adrenal steroids were particularly resistant to insulin, requiring very large doses of this hormone to overcome glycosuria (18). The altered glucose and insulin tolerance of patients with Cushing's syndrome has also been interpreted in this fashion (14). Additional evidence that stressed individuals may exhibit insulin resistance was recently presented by Olsen and Nuetzel who reported that there was a good negative correlation between the severity of illness and the fall in blood sugar after insulin exhibited by patients during insulin tolerance tests (19). In the experiments described in the previous section, the possibility of relative insulin resistance was considered in ill patients and in normal subjects who were treated chronically with cortisone. In all these groups, no fall in blood sugar was noted after the administration of insulin and glucose in contrast to the control normal group and the normals receiving cortisone acutely. In these latter groups, there was a prompt fall in the blood sugar. The following experiments were performed in order to make quantitative measurements of the inhibi-

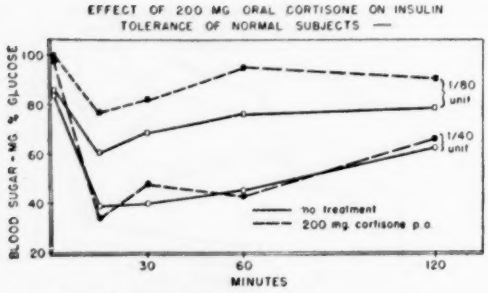

FIG. 8. EFFECT OF 200 MG. OF CORTISONE ON SUBSEQUENT TOLERANCE TO 1/80 AND 1/40 OF A UNIT OF INSULIN PER KILOGRAM INTRAVENOUSLY FOUR HOURS LATER IN NORMAL SUBJECTS

There were ten subjects each in the 1/80 unit group and six controls and four treated subjects in the 1/40 unit group.

tory effects of cortisone on the response to insulin of normal and ill individuals.

For this purpose, tolerance tests were performed with four dose levels of insulin: 1/80, 1/60, 1/40, and 1/10 unit per kilogram intravenously. Figure 8 illustrates the responses of normal subjects to two dose levels of insulin, 1/40 and 1/80 of a unit per kilogram. In neither case, did pretreatment with cortisone modify the magnitude of the fall in blood sugar level. However, with the smallest dose of insulin all blood sugar levels were significantly higher in the cortisone treated group. This might be interpreted as evidence for some degree of insulin resistance following cortisone.

The relative ineffectiveness of cortisone in preventing the fall in blood sugar after insulin, even when the latter substance was administered in relatively small quantities, was surprising, especially in view of the earlier acute experiments which showed that cortisone was capable of altering the response to glucose. To assess the possibility that chronic cortisone treatment might interfere more definitely with the response to insulin in normal subjects, a group of seven subjects was treated with 50 mg. of cortisone every six hours around the clock for seven days. Figure 9 illustrates the results. Insulin tolerance tests, using 1/60 of a unit per kilogram intravenously, were done the day before treatment was initiated, on the second and seventh day of treatment and 24 hours after cortisone was discontinued. Although cortisone treatment was associated with a marked alteration of the response to simultaneously administered glucose and insulin (Figures 6 and 7) in the same subjects on the sixth day of treatment, the response to insulin alone was very little modified by cortisone treatment. The absolute fall in blood sugar was greater in the cortisone treated subjects and the level to which the blood sugar fell was the same as in the untreated group. In the tolerance tests done 24 hours after cortisone had been discontinued, during a period when one might expect relative adrenal insufficiency, the total fall in blood sugar after insulin was not significantly altered. However, the fasting level was lower and consequently the level reached after insulin was significantly lower than in the control or cortisone treated groups, a response which may be interpreted as indicating an increased sensitivity to insulin.

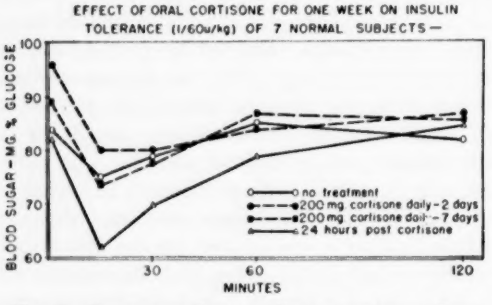

FIG. 9. EFFECTS OF CHRONIC TREATMENT WITH CORTISONE ACETATE ON THE INSULIN TOLERANCE TEST (1/60 UNIT PER KILOGRAM INTRAVENOUSLY) IN NORMAL HEALTHY SUBJECTS

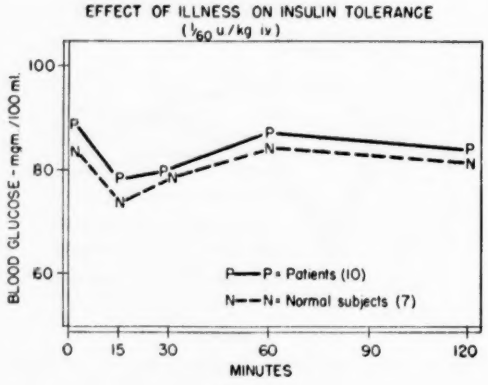

FIG. 10

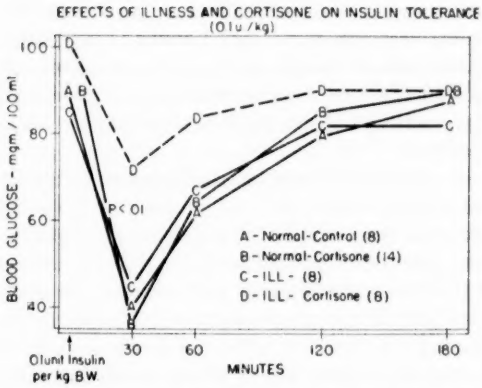

FIG. 11. INSULIN RESISTANCE (1/10 UNIT PER KILOGRAM INTRAVENOUSLY) IN ILL SUBJECTS PRETREATED WITH 200 MG. OF CORTISONE FOUR HOURS PREVIOUSLY AS COMPARED WITH NORMAL INSULIN SENSITIVITY IN UNTREATED ILL SUBJECTS AND NORMAL SUBJECTS PRETREATED WITH CORTISONE

To determine if illness would alter the response to small doses of insulin, as has been reported (19), a group of ten patients with organic illness was tested. The dose of insulin used was 1/60 unit per kilogram. The results are demonstrated in Figure 10, where the effect of illness on insulin tolerance is compared with the response of seven normal subjects. As can be seen, the response to insulin was the same in the ill group as in the untreated normal subjects. Illness, thus, was not associated with any alteration in the response to insulin under the conditions of this experiment.

It was only when cortisone treatment was superimposed on illness that any really appreciable insulin insensitivity was demonstrable (Figure 11). Insulin tolerance tests were performed on seven ill subjects, using in this instance 1/10 unit per kilogram of insulin. This same group of seven patients was pretreated with 200 mg. of cortisone and the insulin tolerance test repeated in the same fashion a few days later. Hypoglycemia developed each time, but the fall noted in the subjects after cortisone was significantly less than that noted when cortisone had not been given. Thus, for the first time in this series of insulin tolerance tests, an impressive degree of insulin intolerance was noted. This result was particularly striking since it occurred in response to a comparatively large dose of insulin.

DISCUSSION

The salient features of this investigation which require comment are as follows: (1) Treatment with cortisone acetate by mouth is followed by an elevation of the fasting blood sugar within four hours which is equally significant in healthy and ill subjects. In normal subjects this is sustained for at least three hours and is not modified by doubling the dose of cortisone. (2) Chronic administration of cortisone is associated with a similar elevation of the fasting blood sugar in normal subjects, but this effect seems to wear off after about seven days. (3) In normal persons, glucose tolerance is impaired by acute cortisone treatment, but insulin tolerance is not notably modified by cortisone treatment when tested with doses of insulin ranging from 1/80 to 1/10 unit per kilogram body weight intravenously, even when cortisone treatment is continued for eight days. When glucose is given simultaneously with insulin to healthy individuals, no abnormality in carbohydrate tolerance is discernible after a single dose of cortisone, but a definite abnormality is apparent after six days of cortisone treatment. (4) The glucose and glucose-insulin tolerance tests yield significantly different results in ill subjects compared with healthy subjects, regardless of whether the normal subjects had been untreated or treated acutely or chronically with cortisone. The response to insulin in the ill subjects in this study, however, was not notably different from that of normal persons. (5) Pretreatment of ill subjects with a single dose of cortisone resulted in a markedly lesser blood sugar fall in the insulin tolerance test than it did in the healthy subjects. Thus, only in the cortisone treated ill subjects was any notable degree of insulin resistance noted.

The finding, that following a single oral dose of cortisone acetate there is an elevated fasting blood sugar and an impairment of glucose tolerance in healthy and ill subjects, is in keeping with well documented observations on the effects of ACTH and cortisone on carbohydrate metabolism in many subjects (20, 21, 22, 23). By itself, it contributes nothing to the understanding of the mechanism by which this response is brought about, *i.e.*, whether by an actual impairment of glucose utilization as in pancreatic diabetes, or by an overproduction of carbohydrate from protein or possibly fat (24);

or finally, whether the higher blood sugar levels simply reflect higher liver glycogen levels such as are known to develop in adrenal hormone treated animals (25). Forsham and Thorn (26) have favored the latter interpretation since they find serum phosphate levels to decrease to a normal degree in ACTH or cortisone treated subjects during the glucose tolerance test. The failure of the fasting sugar levels to remain elevated after seven days of cortisone ingestion is of note. It probably represents an adaptation to the metabolic effect of cortisone, perhaps through the mediation of other hormones, such as insulin.

The abnormality in glucose tolerance in ill subjects likewise is indicative of an alteration in carbohydrate metabolism well-known to occur in response to stressful stimuli (9, 27, 28, 29). Hayes and Brandt (27) have shown that despite the impaired glucose tolerance curves seen in postoperative patients, the fall in serum phosphate is normal, a response which is comparable to that reported by Forsham and Thorn in subjects receiving ACTH or cortisone (26). In our experiments, the shape of the glucose tolerance curve of ill subjects differed in one important respect from that in response to cortisone. In the former, the fasting and three hour blood sugar levels were always normal while in the latter, they were significantly elevated. This difference by itself cannot be accepted as definite evidence that the changes in carbohydrate tolerance after stress and cortisone treatment are dependent on different mechanisms, although it suggests that such might be the case.

The observation that neither illness nor acute or chronic treatment of normal subjects with cortisone modified insulin tolerance to any notable degree was unexpected. Only with the smallest dose of insulin tested, *i.e.*, $\frac{1}{80}$ unit per kilogram, was any degree of insulin resistance noted in healthy subjects receiving cortisone. This was apparent only in the fact that while the absolute fall in blood sugar was identical in the treated and untreated groups, the level to which the blood sugar fell in the cortisone treated group was significantly higher than in the untreated group. Since the observations of Fraser, Albright, and Smith (14) on glucose and insulin tolerance in adrenal disorders and of Ingle, Sheppard, Evans, and Kuizenga (18) on the production with ACTH or cortisone of an insulin resistant type of diabetes in the rat, it has been accepted without question that insulin insensitivity is a characteristic feature of the response to adrenal hormone overdosage. During illness, insulin resistance has also been described (19), but this depends to some extent on the severity of illness and has not been universally confirmed (30).

In the present study, a notable degree of insulin insensitivity was elicited only under certain special circumstances. Firstly, it was noted when glucose was administered simultaneously with the insulin to normal controls given cortisone for six days (but not after a single dose of cortisone) or to ill subjects, with or without a previous dose of cortisone. These experimental conditions correspond roughly to those in the report of Fraser, Albright, and Smith (14) in which insulin insensitivity in Cushing's syndrome was brought out by the glucose-insulin tolerance test, and to Ingle's work in which the amount of insulin necessary to overcome glycosuria in force-fed rats was used as the criterion of insulin resistance (18). In neither case was insulin sensitivity tested in the fasted state. Similarly, in Conn's report of diabetes in normal subjects receiving ACTH, the interpretation of insulin insensitivity was based on the occurrence of glycosuria and impaired glucose tolerance in a single subject who received protamine zinc insulin and ACTH daily, no test of insulin sensitivity being made during fasting.

The second circumstance under which insulin insensitivity was elicited directly was following a single dose of cortisone in ill subjects. This finding has special significance with regard to the interrelationship between stress and the adrenal cortex which will be discussed below. It makes the interpretation of insulin insensitivity in Cushing's syndrome or in animals receiving large doses of cortisone more complex since both these situations are associated with a high incidence of infection and other complications which superimpose a stress factor on the adrenocortical hormone overdosage effect. The results of the present experiments suggest that the mildest degrees of insulin insensitivity may only be readily apparent in the presence of a glucose load, and that in this respect the glucose-insulin tolerance may be a more sensitive indicator of insulin sensitivity than the insulin tolerance test.

Further evidence that the hypoglycemic response to insulin in the fasting state may not necessarily be greatly modified by ACTH or corti-

sone was found in studies on two patients with panhypopituitarism. In these patients, continuous intravenous ACTH or intramuscular cortisone therapy had comparatively little effect on the blood sugar fall after insulin, but caused a marked improvement in blood sugar rise after oral glucose at 30 minutes in the insulin-glucose tolerance test (31, 32).

Taken as a whole, these observations in man may be considered as complementary to those previously described in animals which indicated an interdependent relationship in metabolism between stress or injury and adrenal hormone secretion. In the animal experiments of Ingle (4, 5, 6, 7, 8) and Engel (11, 12, 13), the adrenal factor was made a constant and the stress a variable. It was found that a constant dose of adrenal hormone enabled the adrenalectomized animals to exhibit the characteristic metabolic response to stress, although there was evidence that an increased amount of hormone might be necessary to sustain it. The present experiments were designed so that the stress (illness) and the level of adrenocortical hormone were varied independently. The data show that in the presence of the stress of illness the organism responds to an increase in circulating adrenal hormone by an impairment in carbohydrate and insulin tolerance which is out of proportion to that seen in ill subjects not treated with cortisone as well as to that exhibited by the normal, cortisone treated subject.

One cannot rule out with absolute certainty the possibility that the difference in response of the healthy and ill subjects is due to the fact that the latter are reflecting the additive effects of the ingested cortisone and a high level of endogenous adrenocortical hormone. Against this interpretation, however, are the facts that neither doubling the dose of cortisone nor chronic administration of comparatively large doses of cortisone for eight days reproduced the pattern of carbohydrate and insulin intolerance of illness, with or without cortisone treatment. As shown in the experiment with dietary restriction in normal subjects, inadequate food intake likewise cannot account for observed differences in response to cortisone.

There are, at the moment, relatively few data in the literature concerning man which give direct support to the concept derived from these studies as well as previous observations in the rat (12) that adrenal hormone overdosage may both "sensitize" the organism to respond metabolically to stressful stimuli as well as magnify the metabolic stress response. This is largely because few attempts have been made to isolate the variables involved in the metabolic response to stress. However, certain observations may be considered to be cogent. Thus, for example, among those who have had extensive experience with the therapeutic use of ACTH and cortisone, it is well recognized that the incidence of "hormone overdosage" effects is much greater when these substances are administered to patients who are quite ill than when they are given to subjects who have minor or localized disease, such as that involving the eye alone (33).

During the course of balance studies on patients with lymphomata receiving hydrocortisone or ACTH, Pearson and Eliel (34) observed a patient who developed an intercurrent infection. Prior to this complication, the balance of nitrogen, phosphate, potassium, sodium and chloride had become relatively stable while receiving hydrocortisone. Immediately on developing the infection, with the dose of hormone kept constant, there occurred a prompt and striking loss of nitrogen, phosphorus and potassium and a retention of sodium and chloride, with *no* significant increase in steroid excretion in the urine (35). Although such data must be interpreted with caution, they are consistent with the concept that in the presence of an adequate and presumably constant amount of adrenal hormone the full range of metabolic and hematologic response to stress may be exhibited.

A number of reports exist which also suggest a greater disturbance in carbohydrate metabolism after ACTH or cortisone under conditions of "stress" as compared with normals, notably in schizophrenia (36), liver disease (37) and pregnancy (38), although in none of these reports are there really adequate control studies. Appel and associates (39) described impaired insulin tolerance after four hours intravenous infusion of ACTH in patients who previously had normal insulin and glucose tolerance tests. Presumably, these were ill subjects. In our own experience, a single dose of ACTH, intramuscularly, four hours before tolerance tests, had approximately the same effect on carbohydrate tolerance in a limited number of ill subjects as did cortisone.

The results of this investigation, as well as the

experimental work of Ingle and others, are difficult to reconcile with the concept that the metabolic abnormalities during stress and illness are direct consequences of hypersecretion of adrenal hormone. Rather, it would appear that the adrenal cortex facilitates, in some way, certain metabolic reactions which are peculiar to the tissue response to injury itself. The mechanism by which the metabolic response to injury is initiated and sustained and the precise role of the adrenal and other hormones in it, however, still remain to be elucidated.

SUMMARY

1. A series of 280 glucose, insulin and glucose-insulin tolerance tests were performed on 150 normal subjects and 53 ill patients in order to compare the effects of cortisone on carbohydrate metabolism in the presence and absence of the stress of illness.

2. In normal subjects, a single oral dose of 200 or 400 mg. of cortisone acetate was followed by a significant elevation of the blood sugar four hours later and impairment of the glucose tolerance test. No significant changes following the same treatment could be detected in the glucose-insulin or insulin tolerance tests with doses of insulin, ranging from $\frac{1}{60}$ to $\frac{1}{10}$ of a unit per kilogram intravenously. With $\frac{1}{80}$ of a unit of insulin per kilogram, the total fall in blood sugar was not modified by cortisone treatment, but the level to which it fell was slightly higher than in the untreated group, indicating slight insulin insensitivity.

3. Chronic treatment of normal subjects with 200 mg. of cortisone daily for eight days did not modify the response to $\frac{1}{60}$ of a unit of insulin per kilogram intravenously, but did abolish the blood sugar fall previously seen when glucose was administered simultaneously with $\frac{1}{10}$ unit of insulin per kilogram intravenously.

4. Ill subjects exhibited impaired glucose and glucose-insulin tolerance tests, but normal insulin tolerance at all dose levels of insulin tested. The shapes of the curves of the impaired glucose and glucose-insulin tolerance tests in the ill subjects were significantly different from those of the normal individuals receiving cortisone.

5. Ill subjects pretreated for four hours with 200 mg. of cortisone-acetate showed significantly greater impairment of glucose tolerance and greater resistance to insulin than did normal subjects acutely or chronically treated with cortisone. Cortisone treated patients exhibited only slightly greater insulin resistance in the glucose-insulin tolerance test than did untreated patients, but markedly greater resistance than did normal subjects receiving cortisone acutely or chronically.

6. These observations are considered to be compatible with the concept that there is an interaction between stress and adrenal hormone in modifying carbohydrate metabolism. They are not reconcilable with the view that the metabolic alterations during stress are direct consequences of adrenal cortical hypersecretion.

ACKNOWLEDGMENT

We are indebted to Drs. Augustus Gibson and Elmer Alpert of the Merck Company, Rahway, New Jersey, for the Cortone and to Dr. Otto Behrens of the Eli Lilly Co., Indianapolis, Indiana, for insulin free of hyperglycemic factor used in these experiments. Thanks are also tendered to the many medical and graduate students who whole-heartedly participated in these experiments as part of a laboratory exercise in Physiology.

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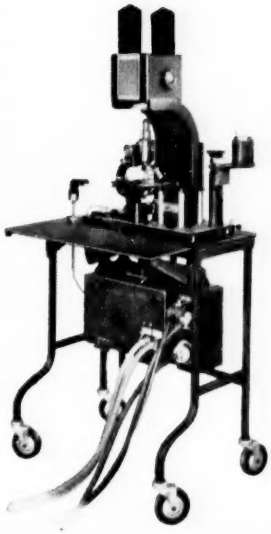

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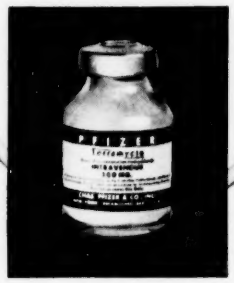

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